

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/15452

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : A61K 31/70, 48/00; C07H 21/04; C12N 9/10 US CL : 424/279.1; 514/23, 44; 536/23.2; 435/183, 193 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 424/279.1; 514/23, 44; 536/23.2; 435/183, 193 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99/49018 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 30 September 1999 (30.09.1999), see entire document.	1-29
Y	BISTRUP ET AL. Sulfotransferases of Two Specificities Function in the Reconstitution of High Endothelial Cell Ligands for L-selectin. J. Cell Biol. 17 May 1999, Vol 145. No. 4, pages 899-910, see entire document.	1-29
Y	LEE ET AL. Cloning and Characterization of a Mammalian N-Acetylglucosamine-6-sulfotransferase That Is Highly Restricted to Intestinal Tissue. Biochem. Biophys. Res. Com. 1999, Vol. 263, pages 543-549, see entire document.	1-29
Y	HIRAOKA ET AL. A Novel, High Endothelial Venule-Specific Sulfotransferase Expresses 6-Sulfo Sialyl LewisX, an L-Selectin Ligand Displayed by CD34. Immunity. July 1999, Vol 11, pages 79-89, see entire document.	1-29
Y	KIMURA ET AL. Reconstitution of functional L-selectin ligands on a cultured human endothelial cell line by cotransfection of alpha 1->3 fucosyltransferase VII and newly cloned GlcNAc-beta:6-sulfotransferase cDNA. Proc. Natl. Acad. Sci. USA. April 1999, Vol. 96, pages 4530-4535, see entire document.	1-29
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
Special categories of cited documents:		
A document defining the general state of the art which is not considered to be of particular relevance	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
B earlier application or patent published on or after the international filing date	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O document referring to an oral disclosure, use, exhibition or other means	*Z*	document member of the same patent family
P document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 27 June 2001 (27.06.2001)	Date of mailing of the international search report 26 JUL 2001	
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Continuation of B. FIELDS SEARCHED Item 3: WEST, MEDLINE, CAPLUS, GENBANK search terms: inventor names, MECA-79, sulfotransferase, L-selectin

261:261-264 (1996)). The homing receptor on lymphocytes is L-selectin, which contains an amino-terminal carbohydrate-binding domain similar to that of the hepatic lectin. Carbohydrate-binding activity of these
5 lectins is calcium-dependent, and they are therefore termed "C-type" lectins (Drickamer, "Molecular Structure of Animal Lectins" in Fukuda and Hindsgaul (Eds), Molecular Glycobiology Oxford University Press: Oxford, U.K. (1994)). Counterreceptors (ligands) on HEV capture
10 circulating lymphocytes via L-selectin-dependent adhesion, leading to transmigration. It has been shown that L-selectin is required for this process (Arbones et al., *supra*, 1994).

The HEV-expressed counterreceptors (ligands)
15 for L-selectin have thus far eluded molecular identification. Consistent with the presence of a C-type lectin domain at the amino terminus of L-selectin, all of the ligands identified to date contain carbohydrate-based recognition determinants. In mouse lymph nodes, two such
20 ligands have been identified as GlyCAM-1 and CD34, both of which are sialomucins (Lasky et al., Cell 69:927-938 (1992); Baumhueter et al., Science 262:436-438 (1993)). CD34 is a type I transmembrane glycoprotein, whereas GlyCAM-1 is a secreted molecule that lacks a
25 transmembrane domain. Additionally, MadCAM-1, which contains a mucin domain in addition to Ig-like domains, can function as a ligand for L-selectin in Peyer's patches (Berg et al., Nature 366:695-698 (1993); and Bargatze et al., Immunity 3:99-108 (1995)). Four human
30 glycoprotein ligands have been biochemically identified, and two of these have been cloned as CD34 and podocalyxin (Berg et al., J. Cell Biol. 114:343-349 (1991); Puri et

al., J. Cell Biol. 131:261-270 (1995); and Sassetti et al., J. Exp. Med. 187:1965-1975 (1998)). All of the human and murine ligands are sialomucin-like, (Puri et al., *supra*, 1995), and CD34 and podocalyxin have a
5 similar overall domain structure (Figure 1) with significant sequence homology in their cytoplasmic domains (Sassetti et al., *supra*, 1998). Notably, only certain glycoforms react with L-selectin. For example, naturally occurring forms of GlyCAM-1, MadCAM-1, CD34 and
10 podocalyxin exist which fail to bind L-selectin due to the absence of necessary post-translational modification (Berg et al., Nature 366:695-698 (1993); Puri et al., *supra*, 1995; Sassetti et al., *supra*, 1998; and Dowbenko et al., J. Clin. Invest. 92:952-960 (1993)). Thus,
15 although CD34 and podocalyxin are widely distributed on vascular endothelium, a limited number of vessels (including HEV) express L-selectin-reactive glycoforms (Sassetti et al., *supra*, 1998; and Baumhueter et al., Blood 84:2554-2565 (1994)).

20 GlyCAM-1 and CD34 were originally identified as L-selectin ligands in extracts of mouse lymph nodes using a recombinant L-selectin/IgG chimera (Lasky et al., *supra*, 1992; Baumhueter et al., *supra*, 1993; and Imai et al., J. Cell Biol. 113:1213-1221 (1991)). Furthermore, a
25 monoclonal antibody, MECA-79, stains HEV in mouse lymph nodes and blocks both lymphocyte attachment to HEV *in vitro* and short-term homing of lymphocytes to lymph nodes *in vivo* (Streeter et al., Nature 331:41-43 (1988)). The MECA-79 monoclonal is remarkable in that it reacts with
30 HEV across a wide range of species including mouse and human (Girard et al., FASEB J. 12:603-612 (1998)). Significantly, MECA-79 and L-selectin/IgG stain the same

complex of glycoproteins in mouse and human lymphoid organs (Sasseti et al., *supra*, 1998; and Hemmerich et al., J. Exp. Med. 180:2219-2226 (1994)). This complex of four or more glycoproteins defined by reactivity with

5 MECA-79 is known as peripheral lymph node addressin (PNAd). Although the structure of the MECA-79 antigen has eluded identification, the epitope is believed to be sulfated (Hemmerich et al., *supra*, 1994) and, in particular, to include a GlcNAc-6-sulfate modification

10 (Kimura et al., Proc. Natl. Acad. Sci. 96:4530-4535 (1999)). Furthermore, previous characterization indicates that the MECA-79 epitope is independent of sialylation and fucosylation (Hemmerich et al., *supra*, 1994; and Maly et al., Cell 86:643-653 (1996)).

15 Nevertheless, the physiologically relevant sulfated structures necessary for L-selectin ligand activity remain to be identified.

L-selectin and its ligands are implicated in lymphocyte recruitment in a variety of chronic

20 inflammatory diseases, and L-selectin ligand activity including MECA-79 expression is induced on microvascular venular endothelium in rheumatoid arthritis, lymphocytic thyroiditis, and inflammatory bowel diseases such as Crohn's disease and ulcerative colitis (Michie et al.,

25 Am. J. Pathol. 143:1688-1698 (1993); and Salmi et al., Gastroenterology 106:596-605 (1994)). Increased MECA-79 expression also is associated with nonobese diabetes in the mouse and with transplant rejection (Hanninen et al., J. Clin. Invest. 92:2509-2515 (1993); and Toppila et al.,

30 Am. J. Pathol. 155:1303-1310 (1999)).

Methods of controlling L-selectin activity would be desirable in order to reduce inflammatory responses mediated by L-selectin. Such methods could be used to treat or prevent conditions such as acute or
5 chronic inflammation; allograft rejection; or tumor metastasis. However, methods of specifically controlling L-selectin activity await elucidation of the sulfated carbohydrate structure on L-selectin ligands, and identification of the enzymes that manufacture the
10 L-selectin ligand carbohydrate determinants.

Thus, there is a need for identification of the L-selectin ligand carbohydrate structure and identification of the enzyme or enzymes that produce this structure. The present invention satisfies this need and
15 provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated β 1,3GnT, or an active fragment
20 thereof, under conditions that allow addition of core 1 GlcNAc linkages to the acceptor molecule, where the β 1,3GnT or active fragment thereof directs expression of a MECA-79 antigen. A β 1,3GnT useful for modifying an acceptor molecule according to a method of the invention
25 can have, for example, substantially the amino acid sequence of human β 1,3GnT (SEQ ID NO: 2) or substantially the amino acid sequence of murine β 1,3GnT (SEQ ID NO: 4).

The invention also provides a method of treating or preventing an L-selectin-mediated condition

in a subject by reducing the expression or activity of a $\beta 1,3\text{GnT}$ that directs expression of a MECA-79 antigen. In a method of the invention, the expression or activity of a $\beta 1,3\text{GnT}$ can be reduced, for example, by administering

5 to a subject an oligosaccharide L-selectin antagonist that inhibits the binding of L-selectin to a MECA-79 antigen. Such an L-selectin antagonist can contain, for example, the oligosaccharide $\text{Gal}\beta 1-4(\text{SO}_3-6)\text{GlcNAc}\beta 1-3\text{Gal}\beta 1-3\text{GalNAc}$ or the oligosaccharide

10 $\text{NeuNAc}\alpha 2-3\text{Gal}\beta 1-4[\text{sulfo-6}(\text{Fuc}\alpha 1-3)\text{GlcNAc}]\beta 1-3\text{Gal}\beta 1-3\text{GalNAc}\alpha 1$, or, in another embodiment, multimers of one or both of these oligosaccharides. In a further embodiment, an L-selectin-mediated condition is treated or prevented by administering to the subject inhibitory

15 antibody material that specifically binds $\beta 1,3\text{GnT}$. In yet a further embodiment, an L-selectin-mediated condition is treated or prevented by administering to the subject a $\beta 1,3\text{GnT}$ antisense nucleic acid molecule that has, for example, at least 20 nucleotides complementary

20 to SEQ ID NO: 1 or SEQ ID NO: 3. In another embodiment, a method of the invention is practiced by reducing the expression or activity of a $\beta 1,3\text{GnT}$ that directs expression of a MECA-79 antigen in combination with reducing the expression or activity of L-selectin

25 sulfotransferase-2 (LSST-2) in the subject.

The present invention also provides an isolated L-selectin antagonist containing an extended core 1 structure which includes the oligosaccharide $\text{Gal}\beta 1-4(\text{SO}_3-6)\text{GlcNAc}\beta 1-3\text{Gal}\beta 1-3\text{GalNAc}$. In a further

30 embodiment, the invention provides an isolated L-selectin antagonist containing the oligosaccharide $\text{NeuNAc}\alpha 2-3\text{Gal}\beta 1-4[\text{sulfo-6}(\text{Fuc}\alpha 1-3)\text{GlcNAc}]\beta 1-3\text{Gal}\beta 1-3\text{GalNAc}$

col. In yet another embodiment, an isolated L-selectin antagonist of the invention contains multimers of one or both the oligosaccharides

Gal β 1-4(SO₃-6)GlcNAc β 1-3Gal β 1-3GalNAc or

5 Gal β 1-4(SO₃-6)GlcNAc β 1-3Gal β 1-3GalNAc.

The present invention also provides an isolated polypeptide which contains an amino acid sequence

encoding a L-selectin sulfotransferase-2 (LSST-2), or an active fragment thereof, that directs expression of a

10 MECA-79 antigen in Chinese hamster ovary (CHO) cells. An isolated polypeptide of the invention can have, for example, substantially the amino acid sequence of human LSST-2 (SEQ ID NO: 6).

15 The present invention further provides substantially purified antibody material that specifically binds a LSST-2 that directs expression of a MECA-79 antigen in CHO cells. Such antibody material, which can be polyclonal or monoclonal antibody material,
20 specifically binds, for example, human LSST-2 having the amino acid sequence SEQ ID NO: 6.

The present invention further provides an isolated nucleic acid molecule which contains a nucleic acid sequence encoding a LSST-2 or an active fragment

25 thereof that directs expression of a MECA-79 antigen in CHO cells. An isolated nucleic acid molecule of the invention can encode, for example, a LSST-2 that has substantially the amino acid sequence of human LSST-2 (SEQ ID NO: 6) and can be, for example, SEQ ID NO: 5.

30 The invention further provides vectors and related host cells that contain a nucleic acid molecule encoding a

LSST-2 or active fragment thereof that directs expression of a MECA-79 antigen in CHO cells. In one embodiment, such a vector is a mammalian expression vector.

The invention also provides an isolated
5 antisense nucleic acid molecule which contains a nucleotide sequence that specifically binds to SEQ ID NO: 5, shown in Figure 4. Such an isolated antisense nucleic acid molecule can have, for example, at least 20 nucleotides complementary to SEQ ID NO: 5. In one
10 embodiment, an isolated antisense nucleic acid molecule contains a nucleotide sequence complementary to the sequence ATG.

Also provided herein is an oligonucleotide, which contains a nucleotide sequence having at least 10
15 contiguous nucleotides of SEQ ID NO: 5, or a nucleotide sequence complementary thereto. An oligonucleotide of the invention can have, for example, at least 15 contiguous nucleotides of SEQ ID NO: 5, or a nucleotide sequence complementary thereto.

20 The present invention also provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated LSST-2, or an active fragment thereof, under conditions that allow addition of a sulfate to a GlcNAc acceptor molecule, where the LSST-2
25 or active fragment thereof directs expression of a MECA-79 antigen in CHO cells. A LSST-2 useful for modifying an acceptor molecule according to a method of the invention can have, for example, substantially the amino acid sequence of human LSST-2 (SEQ ID NO: 6) or an
30 active fragment thereof.

The invention also provides a method of treating or preventing an L-selectin-mediated condition in a subject by reducing the expression or activity of a LSST-2 that directs expression of a MECA-79 antigen in
5 CHO cells. In one embodiment, an L-selectin-mediated condition is treated or prevented by administering to the subject inhibitory antibody material that specifically binds LSST-2. In another embodiment, an
10 L-selectin-mediated condition is treated or prevented by administering to the subject a LSST-2 antisense nucleic acid molecule that has, for example, at least 20 nucleotides complementary to SEQ ID NO: 5.

The invention also provides an isolated polypeptide that contains an amino acid sequence encoding
15 substantially the amino acid sequence of intestinal GlcNAc 6-sulfotransferase (I-GlcNAc6ST) or an active fragment thereof. Such a polypeptide of the invention can have, for example, substantially the amino acid sequence of SEQ ID NO: 8.

20 In addition, the invention also provides substantially purified antibody material that specifically binds an isolated polypeptide having an amino acid sequence encoding substantially the amino acid
25 sequence of I-GlcNAc6ST or an active fragment thereof. Such antibody material, which can be polyclonal or monoclonal antibody material, specifically binds, for example, I-GlcNAc6ST having the amino acid sequence SEQ ID NO: 8.

30 The present invention further provides an isolated nucleic acid molecule which contains a nucleic

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acid sequence encoding an I-GlcNAc6ST or an active fragment thereof. An isolated nucleic acid molecule of the invention can encode, for example, an I-GlcNAc6ST having substantially the amino acid sequence of murine I-GlcNAc6ST (SEQ ID NO: 8) and can be, for example, SEQ ID NO: 7. The invention further provides vectors and related host cells that contain a nucleic acid molecule encoding an I-GlcNAc6ST or active fragment thereof. In one embodiment, such a vector is a mammalian expression vector.

The invention also provides an isolated antisense nucleic acid molecule which contains a nucleotide sequence that specifically binds to SEQ ID NO: 7, shown in Figure 10. Such an isolated antisense nucleic acid molecule can have, for example, at least 20 nucleotides complementary to SEQ ID NO: 7. In one embodiment, an isolated antisense nucleic acid molecule contains a nucleotide sequence complementary to the sequence ATG.

Also provided herein is an oligonucleotide, which contains a nucleotide sequence having at least 10 contiguous nucleotides of SEQ ID NO: 7, or a nucleotide sequence complementary thereto. An oligonucleotide of the invention can have, for example, at least 15 contiguous nucleotides of SEQ ID NO: 7, or a nucleotide sequence complementary thereto.

The present invention also provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated I-GlcNAc6ST, or an active

fragment thereof, under conditions that allow addition of a sulfate to a GlcNAc acceptor molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a model of lymph node HEV
5 ligands for L-selectin. Four sialomucins recognized by MECA-79 are shown. GlyCAM-1, CD34, and Sgp200 have been identified in mouse lymph node. CD34, podocalyxin and Sgp200 have been identified in human tonsils. The complex, defined by purification with MECA-79, is denoted
10 the peripheral lymph node addressin (PNAd). The cDNA encoding Sgp200 (sulfated glycoprotein of 200 kd) has yet to be cloned. White circles designate posttranslational modifications including sialylation, fucosylation, and sulfation. CD34 and podocalyxin share the same overall
15 structural organization, each having an amino-terminal mucin domain, a presumed globular domain with cysteines, a transmembrane domain, and homologous cytoplasmic tails.

Figure 2 shows the human β 1,3GnT nucleotide sequence (SEQ ID NO: 1) and predicted amino acid sequence
20 (SEQ ID NO: 2).

Figure 3 shows the murine β 1,3GnT nucleotide sequence (SEQ ID NO: 3) and predicted amino acid sequence (SEQ ID NO: 4).

Figure 4 shows the human L-selectin
25 sulfotransferase-2 (hLSST-2) nucleotide sequence (SEQ ID NO: 5) and predicted amino acid sequence (SEQ ID NO: 6).

Figure 5 shows a CLUSTALW alignment of mouse β 3GalT-I, -II, -III and -IV and mouse β 3GnT proteins. Conserved residues are shaded. White arrows mark the positions of the cysteine residues conserved among β 3GalT proteins. The black arrow shows the position of the cysteines conserved in the five proteins.

Figure 6 shows *in vitro* substrate specificity of human β 1,3GnT.

Figure 7 shows MECA-79 staining of transfected CHO/CD34 cells.

Figure 8 shows the results of a rolling experiment performed with four stably transfected CHO cell lines. Open circles represent the CHO/CD34/FT7/hLSST-2 cell line. Open squares represent the CHO/CD34/FT7/hLSST-2/C2GnT-L cell line. Filled squares represent the CHO/CD34/FT7/hLSST-2/core 1 extension β 1,3GnT cell line. Filled circles represent the CHO/CD34/FT7/hLSST-2/C2GnT-L/core 1 extension β 1,3GnT line cell.

Figure 9 shows inhibition of anti-MECA-79 antibody binding to MECA-79-reactive CD34 chimeric proteins by synthetic oligosaccharides including sialylated and sialylated, fucosylated forms of the extended core 1 structure.

Figure 10 shows the murine intestinal-GlcNAc 6-sulfotransferase (I-GlcNAc6ST) nucleotide sequence (SEQ ID NO: 7) and predicted amino acid sequence (SEQ ID NO: 8).

DETAILED DESCRIPTION OF THE INVENTION

Lymphocyte homing is important for the surveillance of foreign pathogens. Extravasation of lymphocytes in peripheral lymph nodes is mediated through L-selectin binding to L-selectin ligands, sulfated sialyl Lewis^x present on high endothelial venules (HEV). Recently cloned L-selectin ligand sulfotransferases (LSST or HEC-GlcNAc6ST) form core 2-based selectin ligand functional in rolling assays (Hiraoka et al., Immunity 11:79-89 (1999), and Bistrup et al., J. Cell. Biol. 145:899-910 (1999)). The expression of LSST is highly restricted to HEV, while the sulfotransferase GlcNAc6ST is more widely present and less specific in acceptor substrate requirement.

Analysis of core 2 GnT-leukocyte type knockout mice has indicated that lymphocyte homing and expression of MECA-79 antigen persist even after the gene for the leukocyte type core 2 GnT has been inactivated (Ellies et al., Immunity 9:881-890 (1998)). Structural analysis of L-selectin ligands in HEV of the knockout mice demonstrated that the major oligosaccharides remaining are based on extended core 1 structure such as NeuNAc α 2-3Gal β 1-4[sulfo-6(Fuc α 1-3)GlcNAc] β 1-3Gal β 1-3GalNAc α 1-R. As disclosed herein, a novel β 1,3-N-acetylglucosaminyl-transferase has been isolated that extends core 1 and forms GlcNAc β 1-3Gal β 1-3GalNAc α 1-R. As further disclosed herein, human L-selectin sulfotransferase-2 (hLSST-2), is unique in the ability to produce, when co-transfected into CHO cells together with β 1,3-GnT, NeuNAc α 2-3Gal β 1-4[sulfo-6(Fuc α 1-3)GlcNAc]

β 1-3Gal β 1-3GalNAc α 1-R, resulting in expression of the MECA-79 epitope. As further disclosed herein, oligosaccharides produced in CHO cells expressing both human β 1,3GnT and human LSST-2 support

- 5 L-selectin-mediated lymphocyte rolling (see Example III). These results demonstrate that 6-sulfo sialyl Lewis X structures on core 1 or core 2 oligosaccharides can serve as L-selectin ligands on high endothelial venules.

- As further disclosed herein in Example IV,
- 10 several synthetic oligosaccharides were compared for the ability to inhibit binding of anti-MECA-79 antibody to the MECA-79 antigen produced in the media of CHO/CD34/FT7/hLSST-2/core 1 β 1,3GnT cells. As shown in Figure 9, only the synthetic oligosaccharide with the
- 15 6-S-extended core 1 structure (Gal β 1-4(SO₃-6) GlcNAc β 1-3Gal β 1-3GalNAc) was able to inhibit antibody binding to MECA-79, defining Gal β 1-4(SO₃-6) GlcNAc β 1-3Gal β 1-3GalNAc as a minimal MECA-79 epitope. Sialylated or sialylated and fucosylated forms of the
- 20 6-sulfo extended core 1 structure (NeuNAc α 2-3Gal β 1-4[Fuc α 1-3(sulfo-6)]GlcNAc β 1-3Gal β 1-3GalNAc α 1-octyl) also were efficient inhibitors of antibody binding (Figure 9). As further disclosed herein, the 6-sulfo group was absolutely required, since
- 25 non-sulfated, extended core 1 did not inhibit MECA-79 antibody binding (Figure 9). In addition, the terminal galactose residue in the N-acetyllactosaminyl core 1 was part of the epitope, since the agalacto form required more than a 10 fold greater concentration to achieve
- 30 equivalent inhibition (Figure 9). An absolute requirement for core 1 structure was also demonstrated, since sulfated N-acetyllactosamine lacking a core 1

structure did not show detectable inhibition (Figure 9). These results indicate that the minimum epitope of MECA-79 is the sulfated, extended core1 structure Gal β 1-4(sulfo-6)GlcNAc β 1-3Gal β 1-3GalNAc α 1-R, and that the sialylated and sialylated, fucosylated forms (6-sulfo sLe^x in extended core 1) retain MECA-79 reactivity.

Thus, the present invention is directed to the long-awaited discovery of the structure and minimum epitope of the L-selectin ligand, MECA-79, and to identification of a β 1,3-N-acetylglucosaminyl transferase (β 1,3GnT) and a human sulfotransferase (hLSST-2) that can produce this ligand when co-expressed in CHO cells. These discoveries provide a basis for diagnosing and treating L-selectin-mediated conditions, including acute and chronic inflammation, transplant rejection and tumor metastasis.

The present invention relates to an isolated polypeptide which contains an amino acid sequence encoding a β 1,3GnT, or an active fragment thereof, that directs expression of a MECA-79 antigen in CHO cells. Such an isolated polypeptide can have, for example, substantially the amino acid sequence of human β 1,3GnT (SEQ ID NO: 2) or substantially the amino acid sequence of murine β 1,3GnT (SEQ ID NO: 4).

The term " β 1,3-N-acetylglucosaminyl-transferase," as used herein, is synonymous with " β 1,3GnT" and means an enzyme that catalyzes the β 1-3 linkage of a N-acetylglucosamine (GlcNAc) residue to an acceptor molecule. A β 1,3GnT useful in the invention is a core 1 extension enzyme and, therefore, catalyzes the

β 1-3 linkage of a GlcNAc residue to the core 1 structure
Gal β 1-3GalNAc-R.

A β 1,3GnT that directs expression of a MECA-79
5 epitope can have, for example, substantially the amino
acid sequence of the human β 1,3GnT shown in Figure 2 as
SEQ ID NO: 2 or substantially the amino acid sequence of
the murine β 1,3GnT shown in Figure 3 as SEQ ID NO: 4.
Human β 1,3GnT polypeptide (SEQ ID NO: 2) is a type II
10 membrane protein of 352 amino acids. Human β 1,3GnT (SEQ
ID NO: 2) shares 66.5% amino acid identity with murine
 β 1,3GnT (SEQ ID NO: 4). Regions highly conserved between
human and murine β 1,3GnT are present, for example, at
amino acids 158 to 245, 263 to 322 and 330 to 361 of SEQ
15 ID NO: 2. As disclosed in Example IB, human β 1,3GnT (SEQ
ID NO: 2) forms the MECA-79 antigen when expressed with
L-selectin ligand sulfotransferase-2 in Chinese hamster
ovary (CHO) cells. Thus, such a β 1,3 GnT is
characterized, in part, by the ability to direct
20 expression of a MECA-79 antigen.

The mouse monoclonal antibody, MECA-79, stains
HEV in mouse lymph nodes and blocks lymphocyte attachment
to HEV *in vitro* as well as short-term homing of
lymphocytes to lymph nodes *in vivo* (Streeter et al.,
25 *supra*, 1988). Furthermore, the MECA-79 monoclonal
antibody reacts with HEV across a variety of species and
stains the same complex of glycoproteins in mouse and
human lymphoid organs (Girard et al., *supra*, 1998;
Sasseti et al., *supra*, 1998; Hemmerich et al. *supra*,
30 1994). Thus, while the carbohydrate-based recognition
determinants on the HEV-expressed L-selectin ligands
CD34, podocalyxin, Sgp200 and GlyCAM-2 remain unknown,

these L-selectin ligands contain the MECA-79 antigen (Hemmerich, *supra*, 1994).

As used herein, the term "MECA-79 antigen" means a carbohydrate-containing epitope that specifically reacts with the MECA-79 monoclonal antibody described in Hemmerich, *supra*, 1994. An exemplary MECA-79 antigen is provided herein as $\text{Gal}\beta 1-4(\text{SO}_3-6)\text{GlcNAc}\beta 1-3\text{Gal}\beta 1-3\text{GalNAc}$. The phrase "directs expression of a MECA-79 antigen" refers to production of a carbohydrate-containing epitope that specifically reacts with the MECA-79 monoclonal antibody. It is understood that an enzyme "directs expression of a MECA-79 antigen" only under the appropriate conditions. Such conditions include availability of a core 1 acceptor molecule and an appropriate donor molecule and further include the presence of one or more additional enzymes. Human $\beta 1,3\text{GnT}$ together with the human sulfotransferase LSST-2, but not other sulfotransferases, directs expression of the MECA-79 antigen in CHO cells.

The invention provides a method of treating or preventing an L-selectin-mediated condition in a subject by reducing the expression or activity of a $\beta 1,3\text{GnT}$ that directs expression of a MECA-79 antigen. If desired, a method of the invention can be practiced by reducing the expression or activity of a $\beta 1,3\text{GnT}$ that directs expression of a MECA-79 antigen in combination with reducing the expression or activity of L-selectin sulfotransferase-2 (LSST-2) in the subject.

As used herein, the term "L-selectin-mediated condition" means any pathology or disorder involving the

L-selectin ligand, MECA-79. Such an L-selectin-mediated condition generally can be, for example, acute or chronic inflammation, allograft rejection, or tumor metastasis. An L-selectin-mediated condition also can be, for

5 example, organ transplant rejection, which is typically accompanied by an influx of lymphocytes into the graft. For example, in a rat model of acute cardiac allograft rejection, Toppila et al. demonstrated the induction of L-selectin ligands including MECA-7 on flat-walled

10 venules and capillaries within rejecting cardiac allograft (Toppila et al., Am. J. Pathol. 155:1303-1310 (1999)). Toppila et al. further observed a correlation between the staining intensity of L-selectin ligands on vessels and the severity of acute rejection of heart

15 allografts in humans. L-selectin-mediated conditions further can include rheumatoid arthritis; inflammatory bowel diseases such as Crohn's disease and ulcerative colitis; inflammatory disorders of the skin such as allergic contact dermatitis, psoriasis and Lichen planus;

20 lymphomas; chronic pneumonia; delayed-type hypersensitivity reactions; diabetes; and hyperplastic thymus, each of which are characterized by expression of MECA-79 in HEV-like vessels (Rosen, Am. J. Pathol. 155:1013-1020 (1999); see, also, Table 1). It is

25 understood that these and other conditions of acute or chronic inflammation, allograft rejection or tumor metastasis can be an "L-selectin-mediated" condition that can be treated according to a method of the invention.

Table 1		
L-selectin-mediated conditions		
Organ	Disease process	Reference
Synovium	Rheumatoid arthritis	Michie et al., <u>Am. J. Path.</u> 143:1688-1698 (1993); Van Dinther-Jansses et al., <u>J. Rheum.</u> 17-11-17 (1990)
Gut	Crohn's disease	Salmi et al., <u>Gastroenterology</u> 106:596-605 (1994); Duijvestijn et al., <u>J. Immunol.</u> 138:713-719 (1987)
Gut	Ulcerative colitis	Salmi et al., <u>Eur. J. Immunol.</u> 22:835-843 (1992)
Skin	Cutaneous sites of inflammation such as allergic contact dermatitis, psoriasis and lichen planus	Michie et al., <i>supra</i> , 1993; Arvilommi et al., <u>Eur. J. Immunol.</u> 26:825-833 (1996)
Skin	Cutaneous lymphomas	Michie et al., <i>supra</i> , 1993
Lung	Chronic interstitial pneumonia	
Skin	Delayed-type hypersensitivity reaction	Mackay et al., <u>Eur. J. Immunol.</u> 22:835-843 (1992)
Pancreas	Diabetes	Hanninen et al., <u>J. Clin. Invest.</u> 92:2509-2515 (1993)
Thymus	Hyperplastic thymus	Michie et al., <u>Am. J. Path.</u> 147:412-421 (1995)

The term "reducing the expression or activity" as used herein to a $\beta 1,3\text{GnT}$, means that the amount of functional $\beta 1,3\text{GnT}$ polypeptide or activity is diminished

in the subject in comparison with the amount of functional β 1,3GnT polypeptide in an untreated subject. Similarly, when used in reference to LSST-2 expression or activity, the term "reduced" means that the amount of functional LSST-2 polypeptide or activity is reduced in the treated subject as compared to an untreated subject. Thus, the term "reduced," as used herein, encompasses the absence of a β 1,3GnT that directs expression of a MECA-79 antigen or a LSST-2, as well as protein expression that is present but reduced as compared to the level of β 1,3GnT or LSST-2 expression in an untreated subject. Furthermore, the term reduced refers to suppressed refers to β 1,3GnT or LSST-2 protein expression that is diminished throughout the entire domain of β 1,3GnT or LSST-2 expression, or to expression that is reduced in some part of the β 1,3GnT or LSST-2 expression domain, provided that expression of the MECA-79 antigen is decreased.

As used herein, the term "reduced" also encompasses an amount of β 1,3GnT or LSST-2 polypeptide that is equivalent to wild type β 1,3GnT or LSST-2 expression, but where the β 1,3GnT or LSST-2 polypeptide has a reduced level of activity. For example, mutations within the catalytic domain of β 1,3GnT or LSST-2 that reduce glucosaminyltransferase activity or sulfotransferase activity, respectively, are encompassed within the meaning of the term "reduced."

The present invention relates, in part, to the use of carbohydrate-based drugs for treatment of an L-selectin-mediated condition such as rheumatoid arthritis, inflammatory bowel disease or diabetes.

Carbohydrate drugs are well known in the art and include, for example, Acarbose, a maltotetrose analog for treatment of diabetes, which acts as a competitive inhibitor of sucrase and α -amylase (Bayer AG; Balfour and
5 McTavish, Drugs 46:1025 (1993)). Other carbohydrate drugs include Relenza™ (GG-167, zanamivir), a sialic acid analog for treatment of influenza which is a selective inhibitor of viral neuramidases (Glaxo Wellcome/Biota; Hayden et al., JAMA 275:295 (1996), and SYNSORB Pk™, an
10 oligosaccharide conjugate for treatment of *E. coli* 0157.H7 infection developed by SYNSORB Biotech. Additional carbohydrate-based drugs are well known in the art (see, for example, Dumitru (Ed.), Polysaccharides in Medicinal Applications Dekker, New York (1996)).

15

In one embodiment, the invention provides a method of treating or preventing an L-selectin-mediated condition in a subject by administering to the subject an oligosaccharide L-selectin antagonist that inhibits the
20 binding of L-selectin to a MECA-79 antigen. Such an L-selectin antagonist can contain, for example, the oligosaccharide Gal β 1-4(SO₃-6)GlcNAc β 1-3Gal β 1-3GalNAc or the oligosaccharide NeuNAc α 2-3Gal β 1-4[sulfo-6(Fuc α 1-3)GlcNAc] β 1-3Gal β 1-3GalNAc α 1 or, in another embodiment,
25 multimers of one or both of these oligosaccharides.

As disclosed herein, the MECA-79 epitope has the structure Gal β 1-4(SO₃-6)GlcNAc β 1-3Gal β 1-3GalNAc and is based on a core 1 structure. As further disclosed herein, an L-selectin ligand contains the MECA-79 related
30 structure NeuNAc α 2-3Gal β 1-4[sulfo-6(Fuc α 1-3)GlcNAc] β 1-3Gal β 1-3GalNAc α 1. The term "core 1," as used herein, means the core structure Gal β 1-3GalNAc-R. In conformance

with accepted carbohydrate and chemical nomenclature, "Gal" means galactose; "GalNAc" means N-acetylgalactosamine; "GlcNAc" means N-acetylglucosamine; "SO₃" means sulfate; and "NeuNAc" means N-acetylneuraminate, also known as sialic acid. "R" can be a serine or threonine residue of a peptide or protein or, for example, an octyl, O-methyl, p-nitrophenol, amino pyridine, or other convenient moiety.

10 The term "oligosaccharide," as used herein, means a linear or branched carbohydrate that consists of from 2 to about 50 monosaccharide units joined by means of glycosidic bonds. The monosaccharide units of an oligosaccharide are polyhydroxy alcohols containing
15 either an aldehyde or a ketone group. An oligosaccharide can have, for example, up to 5, 10, 20, 30, 40 or 50 monosaccharide units. It is understood that "an oligosaccharide L-selectin antagonist" may have other non-carbohydrate components in addition to its
20 carbohydrate component.

 An L-selectin antagonist also can be a glycoconjugate or glycomimetic based on the structure Gal β 1-4(SO₃-6)GlcNAc β 1-3Gal β 1-3GalNAc or NeuNAc α 2-3Gal β 1-4[sulfo-6(Fuc α 1-3)GlcNAc] β 1-3Gal β 1-3GalNAc α 1.
25 Thus, an L-selectin antagonist of the invention can be a synthetic glycoconjugate or glycomimetic that retains the ability to inhibit binding of L-selectin to a MECA-79 antigen (Yarema and Bertozzi, Curr. Opin. Chem. Biol. 2:49-61 (1998); Dumitru, *supra*, 1996). Multivalent
30 glycoconjugates are particularly useful L-selectin antagonists of the invention.

As disclosed herein, the MECA-79 epitope is formed, in part, by a core 1 extension enzyme (β 1,3GnT) which catalyzes the β 1-3 linkage of a GlcNAc residue to the core 1 structure ($\text{Gal}\beta$ 1-3 GalNAc-R) and has the

5 structure $\text{Gal}\beta$ 1-4(SO_3 -6) $\text{GlcNAc}\beta$ 1-3 $\text{Gal}\beta$ 1-3 GalNAc . Based on this discovery, the present invention provides an oligosaccharide L-selectin antagonist containing an extended core 1 structure which includes the oligosaccharide $\text{Gal}\beta$ 1-4(SO_3 -6) $\text{GlcNAc}\beta$ 1-3 $\text{Gal}\beta$ 1-3 GalNAc . In

10 one embodiment, an isolated L-selectin antagonist contains the oligosaccharide $\text{NeuNAc}\alpha$ 2-3 $\text{Gal}\beta$ 1-4[sulfo-6($\text{Fuc}\alpha$ 1-3) GlcNAc] β 1-3 $\text{Gal}\beta$ 1-3 $\text{GalNAc}\alpha$ 1. In another embodiment, an L-selectin antagonist contains multimers of one or both of the oligosaccharides

15 $\text{Gal}\beta$ 1-4(SO_3 -6) $\text{GlcNAc}\beta$ 1-3 $\text{Gal}\beta$ 1-3 GalNAc and $\text{NeuNAc}\alpha$ 2-3 $\text{Gal}\beta$ 1-4[sulfo-6($\text{Fuc}\alpha$ 1-3) GlcNAc] β 1-3 $\text{Gal}\beta$ 1-3 $\text{GalNAc}\alpha$ 1. In addition to the structural features set forth above, an L-selectin antagonist inhibits L-selectin activity, for example, by competing for binding to

20 physiological L-selectin ligand. L-selectin antagonists also include variants of these structures which cannot accept a GlcNAc residue at the 3 position of galactose, such as structures in which C-3 of galactose is deoxy; or variants in which GlcNAc contains a 6-dehydro group.

25 Other L-selectin antagonists can be core 1 structure derivatives which cannot accept a GlcNAc residue at the 3 position of galactose.

In a further embodiment, an L-selectin-mediated condition is treated or prevented by administering to the

30 subject inhibitory antibody material that specifically binds β 1,3GnT. In yet a further embodiment, an L-selectin-mediated condition is treated or prevented by

administering to the subject a β 1,3GnT antisense nucleic acid molecule that has, for example, at least 20 nucleotides complementary to SEQ ID NO: 1 or SEQ ID NO: 3.

5 The present invention also provides an isolated polypeptide which contains an amino acid sequence encoding a L-selectin sulfotransferase-2 (LSST-2), or an active fragment thereof, that directs expression of a MECA-79 antigen in Chinese hamster ovary (CHO) cells. An
10 isolated polypeptide of the invention can have, for example, substantially the amino acid sequence of human LSST-2 (SEQ ID NO: 6).

As used herein, the term "isolated" means a polypeptide or nucleic acid molecule that is in a form
15 that is relatively free from contaminating lipids, polypeptides, nucleic acids or other cellular material normally associated with the nucleic acid molecule or polypeptide in a cell.

A LSST-2 polypeptide can have substantially the
20 amino acid sequence of SEQ ID NO: 6. Thus, an LSST-2 polypeptide of the invention can be the naturally occurring human LSST-2 (SEQ ID NO: 6), or a related polypeptide having substantial amino acid sequence similarity to this sequence. Such a related polypeptide
25 typically exhibits greater sequence similarity to human LSST-2 than to other sulfotransferases such as murine LSST, and includes isotype variants, alternatively spliced forms and species homologs of the amino acid sequence shown in Figure 4. As used herein, the term
30 "LSST-2" generally describes polypeptides having an amino

acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 80%, 90%, 95%, 97%, or 99% amino acid sequence identity with SEQ ID NO: 6, said amino acid identity determined with CLUSTALW using the BLOSUM 62 matrix with default parameters, provided that such a polypeptide is able to produce the MECA-79 antigen when expressed in CHO cells under the appropriate conditions. The previously described murine polypeptide, LSST (Hiraoka et al., *supra*, 1999), which is not able to form the MECA-79 antigen when co-transfected into CHO cells with h β 1,3GnT, therefore is not a LSST-2 polypeptide of the invention.

The present invention also provides active fragments of a LSST-2 polypeptide. As used herein, the term "active fragment" means a polypeptide fragment having substantially the amino acid sequence of a portion of a LSST-2 that directs expression of a MECA-79 antigen in CHO cells, provided that the fragment retains the sulfotransferase activity of the parent polypeptide as well as the ability to direct expression of a MECA-79 antigen in CHO cells. An active fragment of LSST-2 can have, for example, substantially the amino acid sequence of a portion of human LSST-2 (SEQ ID NO:6). Sulfotransferase activity can be assayed, for example, as described in Hiraoka et al., *Immunity* 11:79-89 (1999). Activity in directing expression of a MECA-79 antigen can be assayed as set forth in Example IB.

As used herein, the term "substantially the amino acid sequence," when used in reference to a LSST-2

polypeptide or an active fragment thereof, is intended to mean a sequence as shown in Figure 4, or a similar, non-identical sequence that is considered by those skilled in the art to be a functionally equivalent amino acid sequence. For example, an amino acid sequence that has substantially the amino acid sequence of a human LSST-2 polypeptide (SEQ ID NO: 6) can have one or more modifications such as amino acid additions, deletions or substitutions relative to the amino acid sequence of SEQ ID NO: 6, provided that the modified polypeptide retains substantially the ability to direct expression of a MECA-79 antigen in CHO cells, as described further below.

Thus, it is understood that limited modifications can be made to a human LSST-2 polypeptide or another polypeptide of the invention (see below), or to an active fragment thereof without destroying its biological function. A modification can be, for example, an addition, deletion, or substitution of one or more conservative or non-conservative amino acid residues; substitution of a compound that mimics amino acid structure or function; or addition of chemical moieties such as amino or acetyl groups. The activity of a modified LSST-2 polypeptide or fragment thereof can be assayed by transfecting an encoding nucleic acid molecule into CHO cells and assaying for expression of MECA-79 as disclosed herein.

A particularly useful modification of a polypeptide of the invention, or fragment thereof, is a modification that confers, for example, increased stability. Incorporation of one or more D-amino acids is a modification useful in increasing stability of a

polypeptide or polypeptide fragment. Similarly, deletion or substitution of lysine can increase stability by protecting against degradation.

5 The present invention also provides substantially purified antibody material that specifically binds a LSST-2 that directs expression of a MECA-79 antigen in CHO cells. Such antibody material, which can be polyclonal or monoclonal antibody material,
10 specifically binds, for example, human LSST-2 having the amino acid sequence SEQ ID NO: 6.

A LSST-2 polypeptide or polypeptide fragment can be useful to prepare substantially purified antibody
15 material that specifically binds a LSST-2 which directs expression of a MECA-79 antigen in CHO cells. Such antibody material can be, for example, substantially purified polyclonal antiserum or monoclonal antibody material. The antibody material of the invention be
20 useful, for example, in determining the level of LSST-2 polypeptide in a subject.

As used herein, the term "antibody material" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as polypeptide fragments
25 of antibodies that retain a specific binding activity for a LSST-2 polypeptide of at least about $1 \times 10^5 \text{ M}^{-1}$. One skilled in the art would know that anti-LSST-2 antibody fragments such as Fab, $F(ab')_2$, and Fv fragments can retain specific binding activity for a LSST-2 polypeptide and,
30 thus, are included within the definition of antibody material. In addition, the term "antibody material," as used herein, encompasses non-naturally occurring

antibodies and fragments containing, at a minimum, one V_H and one V_L domain, such as chimeric antibodies, humanized antibodies and single chain Fv fragments (scFv) that specifically bind a LSST-2 polypeptide. Such

5 non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, produced recombinantly or obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by

10 Borrebaeck (Ed.), Antibody Engineering (Second edition) New York: Oxford University Press (1995)).

Antibody material "specific for" a LSST-2 polypeptide, or that "specifically binds" a LSST-2 polypeptide, binds with substantially higher affinity to

15 that polypeptide than to an unrelated polypeptide. The substantially purified antibody material of the invention also can bind with significantly higher affinity to a LSST-2 that directs expression of a MECA-79 antigen in CHO cells than to another sulfotransferase that does not

20 direct expression of a MECA-79 antigen in CHO cells.

Anti-LSST-2 antibody material can be prepared, for example, using a LSST-2 fusion protein or a synthetic peptide encoding a portion of a LSST-2 polypeptide such as SEQ ID NO: 6 as an immunogen. One skilled in the art

25 would know that purified LSST-2 polypeptide, which can be produced recombinantly, or fragments of LSST-2, including peptide portions of LSST-2 such as synthetic peptides, can be used as an immunogen. Non-immunogenic fragments or synthetic peptides of LSST-2 can be made immunogenic

30 by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin

(KLH). In addition, various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art are described, for example, by Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1988)).

The term "substantially purified," as used herein in reference to antibody material, means that the antibody material is substantially devoid of polypeptides, nucleic acids and other cellular material. which with an antibody is normally associated in a cell. The claimed antibody material that specifically binds an LSST-2 further is substantially devoid of antibody material of unrelated specificities, i.e. that does not specifically bind a LSST-2. The antibody material of the invention can be prepared in substantially purified form, for example, by LSST-2 affinity purification of polyclonal anti-LSST-2 antisera, by screening phage displayed antibodies against a LSST-2 polypeptide such as SEQ ID NO: 6, or as monoclonal antibodies prepared from hybridomas.

The present invention further provides an isolated nucleic acid molecule which contains a nucleic acid sequence encoding a LSST-2 or an active fragment thereof that directs expression of a MECA-79 antigen in CHO cells. An isolated nucleic acid molecule of the invention can encode, for example, a LSST-2 that has substantially the amino acid sequence of human LSST-2 (SEQ ID NO: 6) and can be, for example, SEQ ID NO: 5. The invention further provides vectors and related host cells that contain a nucleic acid molecule encoding a LSST-2 or active fragment thereof that directs expression

of a MECA-79 antigen in CHO cells. In one embodiment, such a vector is a mammalian expression vector.

The term "nucleic acid molecule" is used broadly to mean any polymer of two or more nucleotides, which are linked by a covalent bond such as a phosphodiester bond, a thioester bond, or any of various other bonds known in the art as useful and effective for linking nucleotides. Such nucleic acid molecules can be linear, circular or supercoiled, and can be single stranded or double stranded DNA or RNA or can be a DNA/RNA hybrid.

A sense or antisense nucleic acid molecule or oligonucleotide of the invention also can contain one or more nucleic acid analogs. Nucleoside analogs or phosphothioate bonds that link the nucleotides and protect against degradation by nucleases are particularly useful in a nucleic acid molecule or oligonucleotide of the invention. A ribonucleotide containing a 2-methyl group, instead of the normal hydroxyl group, bonded to the 2'-carbon atom of ribose residues, is an example of a non-naturally occurring RNA molecule that is resistant to enzymatic and chemical degradation. Other examples of non-naturally occurring organic molecules include RNA containing 2'-aminopyrimidines, such RNA being 1000x more stable in human serum as compared to naturally occurring RNA (see Lin et al., Nucl. Acids Res. 22:5229-5234 (1994); and Jellinek et al., Biochemistry 34:11363-11372 (1995)).

Additional nucleotide analogs also are well known in the art. For example, RNA molecules containing

2'-O-methylpurine substitutions on the ribose residues and short phosphorothioate caps at the 3'- and 5'-ends exhibit enhanced resistance to nucleases (Green et al., Chem. Biol. 2:683-695 (1995)). Similarly, RNA containing

5 2'-amino- 2'-deoxypyrimidines or 2'-fluoro- 2'-deoxypyrimidines is less susceptible to nuclease activity (Pagratis et al., Nature Biotechnol. 15:68-73 (1997)). Furthermore, L-RNA, which is a stereoisomer of naturally occurring D-RNA, is resistant to nuclease

10 activity (Nolte et al., Nature Biotechnol. 14:1116-1119 (1996); Klobmann et al., Nature Biotechnol. 14:1112-1115 (1996)). Such RNA molecules and methods of producing them are well known and routine (see Eaton and Piekern, Ann. Rev. Biochem. 64:837-863 (1995)). DNA molecules

15 containing phosphorothioate linked oligodeoxynucleotides are nuclease resistant (Reed et al., Cancer Res. 50:6565-6570 (1990)). Phosphorothioate-3' hydroxypropylamine modification of the phosphodiester bond also reduces the susceptibility of a DNA molecule to

20 nuclease degradation (see Tam et al., Nucl. Acids Res. 22:977-986 (1994), which is incorporated herein by reference). Furthermore, thymidine can be replaced with 5-(1-pentynyl)- 2'-deoxoridine (Latham et al., Nucl. Acids Res. 22:2817-2822 (1994)). It is understood that

25 nucleic acid molecules, including antisense molecules and oligonucleotides, containing one or more nucleotide analogs are encompassed by the invention.

The invention also provides vectors containing a nucleic acid molecule encoding a LSST-2. Such vectors

30 can be cloning vectors or expression vectors and provide a means to transfer an exogenous nucleic acid molecule into a host cell, which can be a prokaryotic or

eukaryotic cell. Contemplated vectors include those derived from a virus, such as a bacteriophage, a baculovirus or a retrovirus, and vectors derived from bacteria or a combination of bacterial and viral sequences, such as a cosmid or a plasmid. The vectors of the invention can advantageously be used to clone or express LSST-2 or an active fragment thereof. Various vectors and methods for introducing such vectors into a host cell are described, for example, in Ausubel et al.,
10 Current Protocols in Molecular Biology John Wiley & Sons, Inc. New York (1999).

In addition to a nucleic acid molecule encoding a LSST-2 or active fragment thereof, a vector of the invention also can contain, if desired, one or more of
15 the following elements: an oligonucleotide encoding, for example, a termination codon or a transcription or translation regulatory element; one or more selectable marker genes, such as an ampicillin, tetracycline, neomycin, hygromycin or zeomycin resistance gene, which
20 is useful for selecting stable transfectants in mammalian cells; one or more enhancer or promoter sequences, which can be obtained, for example, from a viral, bacterial or mammalian gene; transcription termination and RNA processing signals, which are obtained from a gene or a
25 virus such as SV40; an origin of replication such as an SV40, polyoma or *E. coli* origin of replication; versatile multiple cloning sites; and one or more RNA promoters such as a T7 or SP6 promoter, which allows for *in vitro* transcription of sense and antisense RNA.

30 In one embodiment, a vector of the invention is an expression vector. Expression vectors are well known

in the art and provide a means to transfer and express an exogenous nucleic acid molecule in a host cell.

Contemplated expression vectors include vectors that provide for expression in a host cell such as a bacterial
5 cell, yeast cell, insect cell, frog cell, mammalian cell or other animal cell. Such expression vectors include regulatory elements specifically required for expression of the DNA in a cell, the elements being located relative to the nucleic acid molecule encoding LSST-2 so as to
10 permit expression thereof. The regulatory elements can be chosen to provide constitutive expression or, if desired, inducible or cell type-specific expression. Regulatory elements required for expression have been described above and include transcription and translation
15 start sites and termination sites. Such sites permit binding, for example, of RNA polymerase and ribosome subunits. A bacterial expression vector can include, for example, an RNA transcription promoter such as the *lac* promoter, a Shine-Delgarno sequence and an initiator AUG
20 codon in the proper frame to allow translation of an amino acid sequence.

Mammalian expression vectors can be particularly useful and can include, for example, a heterologous or homologous RNA transcription promoter for
25 RNA polymerase binding, a polyadenylation signal located downstream of the coding sequence, an AUG start codon in the appropriate frame and a termination codon to direct detachment of a ribosome following translation of the transcribed mRNA. Commercially available mammalian
30 expression vectors include pSI, which contains the SV40 enhancer/promoter (Promega; Madison, WI); pTarget™ and pCI, which each contain the cytomegalovirus (CMV)

enhancer/promoter (Promega); pcDNA3.1, a CMV expression vector (Invitrogen; Carlsbad, CA); and pRc/RSV, which contains Rous sarcoma virus (RSV) enhancer/promoter sequences (Invitrogen). In addition to these
5 constitutive mammalian expression vectors, inducible expression systems are available, including, for example, an ecdysone-inducible mammalian expression system such as pIND and pVgRXR from Invitrogen. These and other mammalian expression vectors are commercially available
10 or can be assembled by those skilled in the art using well known methods. An example of a eukaryotic expression vector of the invention is pcDNA1.1/LSST-2, described in Example II below.

The invention also provides a host cell
15 containing a vector that includes a nucleic acid molecule encoding a LSST-2 or an active fragment thereof. Such a host cell can be used to replicate the vector and, if desired, to express and isolate substantially pure recombinant LSST-2 using well known biochemical
20 procedures (see Ausubel, *supra*, 1999). In addition, a host cell of the invention can be used in an *in vitro* or *in vivo* method to transfer sulfate to an acceptor molecule. Such host cells can be chosen or transfected to additionally co-express one or more additional enzymes
25 involved in oligosaccharide biosynthesis, for example, the core 1 extension enzyme, h β 1,3GnT. Such host cells can be used to prepare ligands having high affinity for the L-selectin glycoprotein receptor.

Host cells expressing LSST-2 or an active
30 fragment thereof also can be used to screen for selective inhibitors of LSST-2 or for agents that selectively react

with a L-selectin ligand. These agents can be administered to a subject to prevent or treat an L-selectin-mediated condition as described further below.

Examples of host cells useful in the invention include bacterial, yeast, frog and mammalian cells. Various mammalian cells useful as host cells include, for example, mouse NIH/3T3 cells, CHO cells, COS cells and HeLa cells. In addition, mammalian cells obtained, for example, from a primary explant culture are useful as host cells. Additional host cells include non-human mammalian embryonic stem cells, fertilized eggs and embryos, which can be routinely used to generate transgenic animals, such as mice, which express the novel LSST-2 of the invention. Transgenic mice expressing LSST-2 can be used, for example, to screen for compounds that enhance or inhibit the MECA-79 producing activity of this enzyme. Methods for introducing a vector into a host including electroporation, microinjection, calcium phosphate, DEAE-dextran and lipofection methods well known in the art (see, for example, Ausubel, *supra*, 1999).

The invention also provides an isolated antisense nucleic acid molecule which contains a nucleotide sequence that specifically binds to SEQ ID NO: 5, shown in Figure 4. Such an isolated antisense nucleic acid molecule can have, for example, at least 20 nucleotides complementary to SEQ ID NO: 5. In one embodiment, an isolated antisense nucleic acid molecule contains a nucleotide sequence complementary to the sequence ATG.

An isolated antisense nucleic acid molecule can be useful to reduce LSST-2 expression, thereby treating or preventing an L-selectin-mediated condition in a subject. Antisense nucleic acid molecules can, for example, reduce mRNA translation or increase mRNA degradation and thereby suppress gene expression (see, for example, Galderisi et al., J. Cell Physiol. 181:251-257 (1999)). Methods of using antisense nucleic acid molecules as therapeutic agents are well known in the art (see Galderisi et al., *supra*, 1999; Alama et al., Pharmacol. Res. 36:171-178 (1997); and Temsamani et al., Biotechnol. Appl. Biochem. 26 (part 2):65-71 (1997))

The skilled artisan will recognize that effective reduction of LSST-2 expression depends upon the antisense nucleic acid molecule having a high percentage of homology with the endogenous LSST-2 locus, for example, the endogenous human locus SEQ ID NO: 5. A nucleic acid molecule encoding human LSST-2 (SEQ ID NO: 5) provided herein is useful in the antisense methods of the invention.

The homology requirement for effective suppression of gene expression using antisense methodology can be determined empirically. In general, a minimum of about 80-90% nucleic acid sequence identity is preferred for effective suppression of LSST-2 expression. More preferably, a nucleic acid molecule that is exactly homologous to the gene to be suppressed is used as an antisense nucleic acid molecule. Both antisense oligonucleotides of 20, 22, 25, 30, 35, 40 or more nucleotides, as well as antisense nucleic acid molecules

is expressed in a vector are contemplated for use in the antisense methods of the invention.

Also provided herein is an oligonucleotide, which contains a nucleotide sequence having at least 10
5 contiguous nucleotides of SEQ ID NO: 5, or a nucleotide sequence complementary thereto. An oligonucleotide of the invention can have, for example, at least 15 contiguous nucleotides of SEQ ID NO: 5, or a nucleotide sequence complementary thereto.

10 Oligonucleotides of the invention can advantageously be used, for example, as primers for PCR or sequencing, as probes for research or diagnostic applications, and in therapeutic applications. An
15 oligonucleotide of the invention can incorporate, if desired, a detectable moiety such as a radiolabel, fluorochrome, luminescent tag, ferromagnetic substance, or a detectable agent such as biotin, and used to detect expression of LSST-2 in a cell or tissue. Those skilled
20 in the art can determine the appropriate length and nucleic acid sequence of a LSST-2 oligonucleotide for a particular application. An oligonucleotide of the invention contains a nucleotide sequence having, for
25 example, at least, 10, 12, 14, 16, 18, 20, 25, 30, 35 or 40 contiguous nucleotides of SEQ ID NO: 5, or a nucleotide sequence complementary thereto.

The present invention also provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated LSST-2, or an active fragment thereof, under conditions that allow addition of a
30 sulfate to a GlcNAc acceptor molecule, where the LSST-2

or active fragment thereof directs expression of a MECA-79 antigen in CHO cells. A LSST-2 useful for modifying an acceptor molecule according to a method of the invention can have, for example, substantially the
5 amino acid sequence of human LSST-2 (SEQ ID NO: 6) or an active fragment thereof. In a method of the invention, an isolated LSST-2 can add a sulfate to the 6-position of GlcNAc.

The term "acceptor molecule," as used herein,
10 refers to a molecule that is acted upon, or "modified," by a protein having enzymatic activity. For example, an acceptor molecule can be a molecule that accepts the transfer of a sulfate due to the sulfotransferase activity of a LSST-2 polypeptide. An acceptor molecule
15 can be in substantially pure form or in an impure form such as in a host cell or cellular extract. An acceptor molecule can be a naturally occurring molecule or a completely or partially synthesized molecule. An acceptor molecule can contain one or more sugar residues
20 prior to modification and can be further modified to contain additional sugar residues. An acceptor molecule useful in the invention contains the core 1 structure (Gal β 1-3GalNAc-R) and can be, for example, CD34 as disclosed herein. Additional acceptor molecules include
25 podocalyxin, Sgp200 and GlyCAM-1.

In one embodiment, the invention provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated LSST-2 or an active fragment thereof in combination with an isolated
30 β 1,3GnT that directs expression of a MECA-79 antigen under conditions that allow addition of core 1 GlcNAc

linkages and sulfate to the acceptor molecule such that a MECA-79 antigen is formed. As disclosed herein, human β 1,3GnT (SEQ ID NO: 2) and human LSST-2 (SEQ ID NO: 6) can be used together to modify a core 1 structure to
5 produce the MECA-79 antigen,
Gal β 1-4(SO₃-6)GlcNAc β 1-3Gal β 1-3GalNAc, in CHO cells.

The invention also provides a method of treating or preventing an L-selectin-mediated condition in a subject by reducing the expression or activity of a
10 LSST-2 that directs expression of a MECA-79 antigen in CHO cells. L-selectin-mediated conditions as well as techniques for reducing the expression or activity of an enzyme such as LSST-2 are described hereinabove.

As further disclosed herein in Example V, the
15 mouse intestinal GlcNAc 6-sulfotransferase can, in combination with a β 1,3GnT, form the MECA-79 antigen in Lec2 cells, but not in CHO cells. In these cells, which are defective in Golgi sialylation, more core 1 extension product is formed by the core 1 extension enzyme,
20 β 1,3GnT. Under these conditions, murine intestinal GlcNAc 6-sulfotransferase (I-GlcNAc6ST) adds enough sulfate to form the MECA-79 antigen. Thus, the invention also provides a novel nucleic acid molecule that contains a nucleic acid sequence encoding substantially the amino
25 acid sequence of I-GlcNAc6ST or an active fragment thereof. An isolated nucleic acid molecule of the invention can encode, for example, substantially the amino acid sequence of SEQ ID NO: 8 and can be, for example, SEQ ID NO: 7. In one embodiment, an isolated
30 nucleic acid molecule of the invention encodes

substantially the amino acid sequence of SEQ ID NO: 8, provided that the nucleic acid molecule is not AI115260.

The invention also provides an isolated
5 polypeptide that contains an amino acid sequence encoding substantially the amino acid sequence of intestinal GlcNAc 6-sulfotransferase (I-GlcNAc6ST) or an active fragment thereof. Such a polypeptide of the invention can have, for example, substantially the amino acid
10 sequence of SEQ ID NO: 8.

An I-GlcNAc6ST polypeptide has substantially the amino acid sequence of SEQ ID NO: 8. Thus, an I-GlcNAc6ST polypeptide of the invention can be the naturally occurring I-GlcNAc6ST (SEQ ID NO: 8), or a
15 related polypeptide having substantial amino acid sequence similarity to this sequence. Such a related polypeptide includes isotype variants, alternatively spliced forms and species homologs of the amino acid sequence shown in Figure 10. As used herein, the term
20 "I-GlcNAc6ST" generally describes polypeptides having an amino acid sequence with greater than about 50% identity, preferably greater than about 60% identity, more preferably greater than about 70% identity, and can be a polypeptide having greater than about 75%, 80%, 85%, 90%,
25 95%, 97%, or 99% amino acid sequence identity with SEQ ID NO: 8, said amino acid identity determined with CLUSTALW using the BLOSUM 62 matrix with default parameters, provided that such a polypeptide is able to produce the MECA-79 antigen when expressed in Lec2 cells under the
30 appropriate conditions. The previously described murine polypeptide, LSST (Hiraoka et al., *supra*, 1999) is not an I-GlcNAc6ST polypeptide of the invention.

The present invention also provides active fragments of an I-GlcNAc6ST polypeptide. As used herein, The term "active fragment," when used in reference to an I-GlcNAc6ST polypeptide, means a polypeptide fragment
5 having substantially the amino acid sequence of a portion of an I-GlcNAc6ST, provided that the fragment retains the 6-sulfotransferase activity of the parent polypeptide as well as the ability to direct expression of a MECA-79 antigen when expressed in Lec2 cells. An active fragment
10 can have, for example, substantially the amino acid sequence of a portion of murine I-GlcNAc6ST (SEQ ID NO:8). Sulfotransferase activity can be assayed, for example, as described in Hiraoka et al., Immunity 11:79-89 (1999). Activity in directing expression of a
15 MECA-79 antigen can be assayed as set forth in Example IB.

Furthermore, the term "substantially the amino acid sequence," when used in reference to an I-GlcNAc6ST
20 polypeptide or an active fragment thereof, is intended to mean a sequence as shown in Figure 10, or a similar, non-identical sequence that is considered by those skilled in the art to be a functionally equivalent amino acid sequence. For example, an amino acid sequence that
25 has substantially the amino acid sequence of an I-GlcNAc6ST polypeptide (SEQ ID NO: 8) can have one or more modifications such as amino acid additions, deletions or substitutions relative to the amino acid sequence of SEQ ID NO: 8, provided that the modified
30 polypeptide retains substantially 6-sulfotransferase activity as well as the ability to direct expression of a MECA-79 antigen in Lec2 cells (see Example V).

In addition, the invention also provides substantially purified antibody material that specifically binds an isolated polypeptide having an amino acid sequence encoding substantially the amino acid sequence of I-GlcNAc6ST or an active fragment thereof. Such antibody material, which can be polyclonal or monoclonal antibody material, specifically binds, for example, murine I-GlcNAc6ST having the amino acid sequence SEQ ID NO: 8. Thus, such antibody material includes polyclonal and monoclonal antibodies, as well as polypeptide fragments of antibodies that retain a specific binding activity for an I-GlcNAc6ST polypeptide of at least about $1 \times 10^5 \text{ M}^{-1}$. As set forth above, such antibody material includes Fab, F(ab')₂, and Fv fragments as well as chimeric and humanized antibodies and single chain Fv fragments (scFv) that specifically bind an I-GlcNAc6ST polypeptide of the invention.

The present invention further provides an isolated nucleic acid molecule which contains a nucleic acid sequence encoding an I-GlcNAc6ST or an active fragment thereof. An isolated nucleic acid molecule of the invention can encode, for example, an I-GlcNAc6ST having substantially the amino acid sequence of murine I-GlcNAc6ST (SEQ ID NO: 8) and can be, for example, SEQ ID NO: 7. The invention further provides vectors and related host cells that contain a nucleic acid molecule encoding an I-GlcNAc6ST or active fragment thereof. In one embodiment, the vector is a mammalian expression vector. As set forth above, a variety of vectors, including cloning and expression vectors, and host cells are well known in the art.

The invention also provides an isolated antisense nucleic acid molecule which contains a nucleotide sequence that specifically binds to SEQ ID NO: 7, shown in Figure 10. Such an isolated antisense
5 nucleic acid molecule can have, for example, at least 20 nucleotides complementary to SEQ ID NO: 7. In one embodiment, an isolated antisense nucleic acid molecule contains a nucleotide sequence complementary to the sequence ATG. An antisense nucleic acid molecule can
10 have, for example, 20, 22, 25, 30, 35, 40 or more nucleotides.

Also provided herein is an oligonucleotide, which contains a nucleotide sequence having at least 10 contiguous nucleotides of SEQ ID NO: 7, or a nucleotide
15 sequence complementary thereto. An oligonucleotide of the invention can have, for example, at least 15 contiguous nucleotides of SEQ ID NO: 7, or a nucleotide sequence complementary thereto.

As set forth above, a sense or antisense
20 nucleic acid molecule or oligonucleotide of the invention is a polymer of two or more nucleotides, which are linked by a covalent bond such as a phosphodiester bond, a thioester bond, or any of various other bonds known in the art as useful and effective for linking nucleotides.
25 Furthermore, a nucleic acid molecule or oligonucleotide of the invention can contain one or more nucleic acid analogs (see above). An oligonucleotide of the invention contains a nucleotide sequence having, for example, at least, 10, 12, 14, 16, 18, 20, 25, 30, 35 or 40
30 contiguous nucleotides of SEQ ID NO: 7, or a nucleotide sequence complementary thereto.

The present invention also provides a method of modifying an acceptor molecule by contacting the acceptor molecule with an isolated I-GlcNAc6ST, or an active fragment thereof, under conditions that allow addition of a sulfate to a GlcNAc acceptor molecule.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

CLONING AND CHARACTERIZATION OF THE HUMAN CORE 1 EXTENSION ENZYME, β 1,3-N-ACETYLGLUCOSAMINYLTRANSFERASE (β 1,3GnT)

This example describes the cloning and characterization of human and murine β 1,3-N-acetylglucosaminyltransferase (β 1,3GnT).

A. Cloning and characterization of human β 1,3GnT

Sequences homologous among β 1,3-galactosyltransferases and β 1,3-N-acetylglucosaminyltransferases shown in Figure 5 (Zhou et al., Proc. Natl. Acad. Sci., USA 96:406-411 (1999)) were used as probes to search dbEST using the tblastn program. An EST clone (AB015630) containing a single open reading frame of 372 amino acids was obtained. Primers 5'-CTGGCTGGCCAGGATGAAGTATCTCC-3' (β 1,3GnT-A1; SEQ ID NO: 9) and 5'-CCTGATGCTGACTCAGTAGATCTGTGTC-3' (β 1,3GnT-A2AS; SEQ ID NO: 10) were designed based on EST AB015630. After amplification of single-stranded cDNA prepared from HT29 cells using the Thermoscript RT-PCR system (Gibco-BRL #11146-024; Baithersburg, MD), a 1.2 kb fragment

containing full-length coding sequence was isolated (see Figure 2). The 1.2 Kb fragment containing the full-length human β 1,3GnT cDNA was subcloned into the mammalian expression vector pcDNA3.1 (Invitrogen) and designated pcDNA3.1/h β 1,3GnT-A.

In order to characterize the human β 1,3GnT enzyme, a soluble form of the enzyme was prepared by amplifying amino acids 44 to 372 with PCR primers 5'-CGGGATCCCGAGGCCCTGGCCTGGCCCACTCC-3' (β 1,3GnT-A-5'Bam; SEQ ID NO: 11) and 5'-GCTCTAGACTCAGTAGATCTGTGTCTGATTGC-3' (β 1,3GnT-A-3'AS-Xba; SEQ ID NO: 12) and subsequently cloning the amplified fragment into the *Bam*HI and *Xba*I sites of pcDNA3.1/HSB, a modified vector based on pcDNA3.1/Hydro (Invitrogen) and containing a signal peptide followed by a 6 histidine tag. This vector (4 μ g) was transfected into Chinese hamster ovary (CHO) cells using lipofectamine PLUS (Gibco-BRL #10964-013). As a negative control, CHO cells were mock transfected with a vector lacking the β 1,3GnT sequence.

Media from cells expressing the soluble enzyme or mock transfected were collected and concentrated essentially as described in Yeh et al., J. Biol. Chem. 274:3215-3221 (1999). For analysis of β 1,3-galactosyltransferase activity, 3 H-UDP-galactose was used as the sugar nucleotide donor and GalNAc- α -pNP and GlcNAc- β -pNP were used as oligosaccharide acceptor molecules. For detection of β 1,3-N-acetylglucosaminyltransferase (β 1,3GnT) activity, 3 H-UDP-GlcNAc was used as the sugar nucleotide donor with the following oligosaccharide acceptor molecules: Gal β 1,3Glc- β -pNP; core 1 pNP (Gal β 1,3GalNAc- α -pNP);

core 2 pNP (Gal β 1,3(GlcNAc β 1,6)GalNAc- α -pNP); Gal- α -pNP and Gal- β -pNP.

Supernatant from cells expressing the soluble enzyme or mock transfected was assayed for *in vitro* enzyme activity. As shown in Figure 6, concentrated medium from soluble enzyme transfected cells was found to have activity in transferring ^3H -UDP-GlcNAc to core 1-pNP and core 2-pNP. These results indicate that the cloned enzyme has activity as a core 1 extension β 1,3-N-acetylglucosaminyltransferase.

B. Production of the MECA-79 antigen using recombinant h β 1,3GnT (SEQ ID NO: 2)

CHO cells were transfected with CD34 and either (a) no enzyme; (b) pcDNA1/hLSST-2 alone; pcDNA3.1/Zeo/m β 1,3GnT alone; or pcDNA1/hLSST-2 and pcDNA3.1/Zeo/m β 1,3GnT together using lipofectamine essentially as described above. Mock transfected and transfected cells were stained with MECA-79 antibody obtained from Pharmingen (San Diego, CA), and further incubated with goat anti-rat IgM antibodies essentially as described in Hemmerich et al., *supra*, 1994. As shown in Figure 7, positive staining with MECA-79 antibody was only observed in cells co-transfected with both hLSST-2 and m β 1,3GnT vectors, but not in cells only transfected with either enzyme alone. No other sulfotransferases examined showed MECA-79 expression when cotransfected into CHO cells with m β 1,3GnT. These results indicate that the human L-selectin sulfotransferase-2 and the core 1 extension enzyme β 1,3GnT are sufficient to form the MECA-79 antigen when co-expressed in CHO cells.

C. Cloning and characterization of murine β 1,3GnT

Several sets of primers based on the human core 1 extension β 1,3GnT were used for PCR amplification of single stranded cDNA prepared from mouse small intestine using a SMART PCR cDNA synthesis kit according to the manufacturer's instructions (Clontech #K1052-1). PCR amplification was performed using the following conditions: 94°C for 2 minutes, followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. Only one set of primers gave a specific amplification product. Primers A7 (5'-TTCCTGCTGCTGGTGATCAAGTCC-3'; SEQ ID NO: 13), which corresponds to human β 1,3GnT nucleotides 335 to 358) and primer A3AS (5'-CAGGACCTGCTTGAGCGTGAGGTTG-3'; SEQ ID NO: 14), which corresponds to human β 1,3GnT nucleotides 560 to 585, gave a product of 251 bp.

5'- and 3'-RACE were performed to isolate additional murine β 1,3GnT sequence. 5'-RACE was performed using Marathon-Ready mouse testis cDNA (Clontech) using mA2AS primer (5'-ATGGAAATCCCACTGGAGAATGTCGCCGT-3' (SEQ ID NO: 15) and the AP1 primer provided by Marathon-Ready cDNA kit. 3'-RACE was performed using mA1 primer (5'-GCCTGCAAACTATGGGCGCCGCCAGAT-3' (SEQ ID NO: 16) and the SMART primer (Clontech) on mouse small intestine single stranded cDNA prepared using Clontech's SMART PCR cDNA synthesis kit as a template. The full-length cDNA was amplified based on the RACE sequence from mouse small intestine single-stranded cDNA and subcloned into pCDNA3.1/Zeo and designated pCDNA3.1/Zeo/m β 13, GnT.

EXAMPLE II

CLONING OF HUMAN L-SELECTIN LIGAND SULFOTRANSFERASE
(LSST-2)

5 This example describes the isolation of a nucleic acid molecule encoding human L-selectin ligand sulfotransferase-2 (LSST-2), which, together with the β 1,3-N-acetylglucosaminyltransferase, directs expression of the MECA-79 antigen.

10 Like other sulfotransferases in the same gene family (Mazany et al., Biochim. Biophys. Acta 1407:92-97 (1998)), the coding sequence for human LSST-2 was expected to reside in a single exon. Thus, human genomic DNA was used as the template for PCR-based cloning.

15 Primers corresponding to nucleotides 891 to 910 and nucleotides 1327-1302 of mouse LSST-1 (Hiraoka et al., *supra*, 1999) were used to amplify human genomic DNA as follows. Samples were denatured for 3 minutes at 94°C, followed by 40 cycles of 1 minute at 94°C, 30 seconds at

20 61°C, and 45 seconds at 72°C. The amplified products were cloned into pBluescript by TA cloning. The resultant coding sequence was 79.2% identical to mouse LSST-1 at the nucleotide level.

 To clone the full-length LSST-2 coding

25 sequence, a P1 phage library of human genomic DNA (Genome System Inc.; St. Louis, MO) was PCR-amplified using primers 5'-CCGAATTCTCCCGAGAACGCACAAAG-3' (SEQ ID NO: 17) and 5'-CCCAAGCTTCTCATAGCGCACAAGCAG-3' (SEQ ID NO: 18). The PCR was carried out for 30 cycles using a 67°C

30 annealing temperature. From the single positive clone, DNA was purified and sequenced directly. The coding

sequence present on the single exon was confirmed by reverse transcriptase (RT)-PCR using poly(A)⁺ RNA isolated from human lymph node, as described previously (Hiraoka et al., *supra*, 1999). Three pairs of primers used in these PCR reactions correspond to

5'-TTGGCCAGAAGGGGAATAG-3' (SEQ ID NO: 19) and
5'-CCACTGAAAGAGGCTGGACTGT-3' (SEQ ID NO: 20);
5'-GGTTCTGTCTTCCTGGCGCTC-3' (SEQ ID NO: 21) and
5'-TTTGGCAGATGACCTGCATCAC-3' (SEQ ID NO: 22); and
10 5'-AGAACGCACAAAGGAGATCTCA-3' (SEQ ID NO: 23) and
5'-AGATGTAGGCAAGGCTCAGAAG-3' (SEQ ID NO: 24). PCR with the first two pairs of primers was performed by denaturation for 3 minutes at 94°C, followed by 35 cycles of 1 minute at 94°C, 30 seconds at 56°C, and 1 minute at
15 72°C. For the PCR with the third pair of primers, the annealing temperature was changed to 55°C. With the first pair of primers (SEQ ID NOS: 19 and 20), the expected characteristic fragment of 470 bp was obtained. With the second pair of primers (SEQ ID NOS: 21 and 22),
20 the expected characteristic fragment of 617 bp was obtained. With the third pair of primers (SEQ ID NOS: 23 and 24), the expected characteristic fragment of 600 bp was obtained.

The cDNA containing full-length coding sequence
25 of human LSST-2 was excised by *Xba*I and *Tfi*II, blunt-ended and cloned into pcDNA1.1 (Invitrogen). The resulting LSST-2 expression vector, in which the LSST-2 coding sequence is expressed under control of the CMV promoter, was designated pcDNA1.1/LSST-2.

EXAMPLE III**FUNCTIONAL ANALYSIS OF HUMAN β 1,3GnT**

This example describes the function of h β 1,3GnT when stably expressed in CHO cells with hLSST-2.

5 The following CHO cell lines were generated by stable transfection: CHO/CD34/FT7/hLSST-2; CHO/CD34/FT7/hLSST-2/C2GnT-L; CHO/CD34/FT7/hLSST-2/core 1 extension β 1,3GnT; and CHO/CD34/FT7/hLSST-2/C2GnT-L/core 1 extension β 1,3GnT.

10 The stable cell lines were established by standard procedures. Cells were selected with a combination of neomycin, hygromycin and zeocin. The expression of each gene was confirmed by immunostaining with specific antibodies against the
15 relevant cell surface antigens.

 Expression of human CD34 was confirmed by the positive staining of cells with anti-human CD34 antibody. CHO/CD34/FT7/hLSST-2 was first established. The expression of human fucosyltransferase 7 (FT7) was
20 confirmed by the positive staining of cells with anti-sialyl Lewis x (product of FT7) antibody 2H5 as described in Kimura et al., Proc. Natl. Acad. Sci., USA 96:4530-4535 (1997). Expression of hLSST-2 was confirmed by transient transfection of β 1,3GnT-A (core 1
25 extension β 1,3GnT) and cells were stained with MECA-79 as described above. For the confirmation of C2GnT expression in the CHO/CD34/FT7/hLSST/C2GnT-L cell line, the NCC-ST-439 antibody against sialyl Lewis x core 2 structure was used essentially as described in Kumamoto

et al., Biochim. Biophys. Res. Comm. 247:514-517 (1998).
For the confirmation of core 1 extension β 1,3GnT
expression in the CHO/CD34/FT7/hLSST/core 1
extension β 1,3GnT cell line, MECA-79 antibody staining
5 was performed as described above.

Cells were grown as a monolayer on tissue
culture flasks, and mouse lymphocytes were allowed to
flow over the monolayer under different shear forces
essentially as described in Fuhlbrigge et al., J. Cell
10 Biol. 135:837-48 (1996). The number of lymphocytes which
rolled on the cell monolayer were monitored by video
camera and counted. As shown in Figure 8, CHO cells
expressing either the core 2 extension enzyme, C2GnT-L
(open square) or the human core 1 extension enzyme,
15 β 1,3GnT (filled square) rolled more than cells only
expressing fucosyltransferase VII (FT7; open circle).
Furthermore, rolling was significantly enhanced when
lymphocytes rolled on cells expressing both the core 2
extension enzyme, C2GnT-L, and human β 1,3GnT (filled
20 circle). These results indicate that both core 1 and
core 2 extended sulfo sialyl Lewis X determinants play a
role in lymphocyte homing.

EXAMPLE IV

DEFINITION OF THE MINIMUM EPITOPE OF THE MECA-79 ANTIGEN

25 This example describes the use of ELISA
analysis to define the minimum epitope of the MECA-79
antigen.

A. ELISA Assays with anti-MECA-79 Antibody

The stable CHO transfectants described in Example III were grown on 10 cm plates and transiently transfected with soluble form of human CD34 (pcDNA3.1/HSH
5 véctor) using LipofectAmine PLUS (GibcoBRL). One day after transfection, the culture media was replaced with 10 ml of OptiMEM reduced-serum medium. After culturing for an additional two days, the culture media was collected. Cell debris was removed by centrifugation,
10 and the media was concentrated 10-fold by Centriprep 10 concentrators.

The concentrated media was diluted 100-fold in Tris-buffered saline (TBS; 50 mM Tris, 150 mM NaCl, pH 7.5). The wells of 96-well polystyrene microtiter
15 plates (Nunc, F96 Maxisorp Cat. # 442404) were coated overnight with 100 µl of diluted media at 4°C. The plates were washed three times with TBS and were blocked with 250 µl of 5% BSA (in TBS) at room temperature for at least two hours (or 4°C overnight). The wells were
20 washed three times with washing buffer (TBS containing 0.1% Tween 20). Two-fold serially diluted MECA-79 antibody at a concentration of 1:200 to 1:12,800 (Pharmingen) was prepared in dilution buffer (5% BSA in washing buffer). Fifty µl of diluted antibodies were
25 added to each well and incubated at room temperature for one hour. After washing three times with washing buffer, 50 µl of anti-rat IgM-alkaline phosphatase conjugate (1:500 in dilution buffer) was added to each well and allowed to incubate at room temperature for one hour.
30 Following washing three times with washing buffer, the wells were washed twice with deionized water. Alkaline

phosphatase substrate p-nitro-phenylphosphate (1 mg/ml) was freshly prepared in bicarbonate buffer (0.1 M NaHCO₃, pH 9.6) containing 0.5 mM MgCl₂. Fifty microliter of this substrate solution was added to each well and allowed to incubate at 37°C. The optical density at 405 nm of each well was recorded using Spectra MAX Plus microtiter plate reader (Molecular Device Corp.). Positive readings were observed from the media of CHO cells harboring both hLSST-2 and core 1 extension β 1,3GnT and CHO/CD34/FT7/hLSST-2/core 1 extension β 1,3GnT/core2GnT).

B. Inhibition of MECA-79 Antibody Binding by Synthetic Oligosaccharides

Synthetic oligosaccharides were mixed at the indicated concentrations with MECA-79 antibody (at a final dilution of 1:10,000). The mixtures were incubated at room temperature for one hour before addition to wells precoated with transfected media from CHO/CD34/FT7/LSST/core 1 extension β 1,3GnT cells as above. Antibody binding was assayed as described above.

The results shown in Figure 9 indicate that only the 6-S-extended core 1 structure (Gal β 1-4(SO₃-6) GlcNAc β 1-3Gal β 1-3GalNAc) was active in inhibiting binding of anti-MECA-79 antibody. Thus, these results define the minimum epitope of MECA-79 as Gal β 1-4(SO₃-6) GlcNAc β 1-3Gal β 1-3GalNAc.

To further confirm and refine the requirements for the MECA-79 reactivity, oligosaccharides derived from the extended core 1 glycan were chemically synthesized and examined for their ability to inhibit MECA-79

antibody binding to MECA-79-reactive CD34 chimeric proteins. MECA-79 antibody binding was efficiently inhibited by a 6-sulfo extended core 1 oligosaccharide, Gal β -4(sulfo-6)GlcNAc β 1-3Gal β 1-3GalNAc α 1-octyl, and its sialylated or sialylated, fucosylated forms, NeuNAc α 2-3Gal β 1-4[Fuc α 1-3(sulfo-6)]GlcNAc β 1-3Gal β 1-3GalNAc α 1-octyl (see Figure 9). The 6-sulfo group was absolutely required, since non-sulfated, extended core 1 oligosaccharide did not inhibit MECA-79 antibody binding (Figure 9). In addition, the terminal galactose residue in the *N*-acetyllactosaminyl core 1 was part of the epitope, since the agalacto form required more than a 10 fold increase concentration to achieve equivalent inhibition (Figure 9). An absolute requirement for core 1 structure was also demonstrated, since sulfated *N*-acetyllactosamine lacking a core 1 structure did not show detectable inhibition. These results are consistent with previous studies showing that sialic acid and fucose are not integral parts of the MECA-79 epitope (Hemmerich et al., *supra*, 1994; Maly et al., *supra*, 1996). The results also are consistent with previous findings that the MECA-79 antibody can inhibit lymphocyte homing without prior removal of sialic acid or fucose (Streeter et al., *supra*, 1988; Clark et al., J. Biol. Chem. 140:721-731 (1998)).

These results demonstrate that the minimum epitope of MECA-79 is the sulfated, extended core 1 structure Gal β 1-4(sulfo-6)GlcNAc β 1-3Gal β 1-3GalNAc α 1-R, and that sialylated and sialylated, fucosylated forms of this structure (6-sulfo sLe^x in extended core 1 forms) retain MECA-79 reactivity.

EXAMPLE V**MURINE INTESTINAL GlcNAc 6-SULFOTRANSFERASE**

This example describes the cloning and characterization of the murine intestinal GlcNAc 6-sulfotransferase.

The coding sequence of mouse LSST-1 (Hiraoka et al., Immunity 11:79-89 (1999)) was used as probe to search dbEST using tblstx program. One unknown query gene (AI115260) was found to have 53.8% identity with the coding regions of mouse LSST-1. AI115260 is a sequence isolated from mouse embryo cDNA. Sequence analysis of this cDNA, obtained from Genome Systems (St. Louis, MS), revealed that this cDNA encodes a protein of 396 amino acids, designated intestinal GlcNAc 6-sulfotransferase.

The cDNA insert was digested with EcoRI and XbaI and cloned into the corresponding sites of pcDNA3.1 (Invitrogen) to produce the expression vector pcDNA3-I-GlcNAc6ST.

Lec2 cells, which are defective in Golgi sialylation due to a CMP-sialic acid transporter defect, were doubly transfected with pcDNA3-I-GlcNAc6ST and pcDNA3.1/h β 1,3GnT-A. Because of the absence of sialic acid in Lec2 cells, core 1 extension occurs with the competition of sialylation and, therefore, more core 1 extended structure is formed by the core 1 extension enzyme β 1,3GnT. Under these conditions, the MECA-79 antigen was produced in the doubly transfected Lec2 cells. Similar production of MECA-79 antigen was observed when Lec2 cells were doubly transfected with mLSST-1 and h β 1,3GnT (SEQ ID NO: 2). These results

indicate that, under certain conditions, mLSST-1 or I-GLCNAc6ST can form the MECA-79 antigen.

All journal article, reference, and patent citations provided above, in parentheses or otherwise,
5 whether previously stated or not, are incorporated herein by reference.

Although the invention has been described with reference to the examples above, it should be understood that various modifications can be made without departing
10 from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

We claim:

1. A method of treating or preventing an L-selectin-mediated condition in a subject, comprising reducing the expression or activity of a $\beta 1,3\text{GnT}$ that directs expression of a MECA-79 antigen.
2. The method of claim 1, comprising administering to said subject an oligosaccharide L-selectin antagonist that inhibits the binding of L-selectin to a MECA-79 antigen.
- 10 3. The method of claim 2, wherein said L-selectin antagonist comprises the oligosaccharide $\text{Gal}\beta 1\rightarrow 4(\text{SO}_3\rightarrow 6)\text{GlcNAc}\beta 1\rightarrow 3\text{Gal}\beta 1\rightarrow 3\text{GalNAc}$.
4. The method of claim 3, wherein said L-selectin antagonist comprises
15 $\text{NeuNAc}\alpha 2\rightarrow 3\text{Gal}\beta 1\rightarrow 4[\text{sulfo}\rightarrow 6(\text{Fuc}\alpha 1\rightarrow 3)\text{GlcNAc}]\beta 1\rightarrow 3\text{Gal}\beta 1\rightarrow 3\text{GalNAc}\alpha 1$.
5. The method of claim 3, wherein said L-selectin antagonist comprises two or more of the oligosaccharide $\text{Gal}\beta 1\rightarrow 4(\text{SO}_3\rightarrow 6)\text{GlcNAc}\beta 1\rightarrow 3\text{Gal}\beta 1\rightarrow 3\text{GalNAc}$.
- 20 6. The method of claim 4, wherein said L-selectin antagonist comprises two or more of the oligosaccharide $\text{NeuNAc}\alpha 2\rightarrow 3\text{Gal}\beta 1\rightarrow 4[\text{sulfo}\rightarrow 6(\text{Fuc}\alpha 1\rightarrow 3)\text{GlcNAc}]\beta 1\rightarrow 3\text{Gal}\beta 1\rightarrow 3\text{GalNAc}\alpha 1$.

7. The method of claim 1, comprising administering to said subject inhibitory antibody material that specifically binds $\beta 1,3\text{GnT}$.

8. The method of claim 1, comprising
5 administering to said subject a $\beta 1,3\text{GnT}$ antisense nucleic acid molecule.

9. The method of claim 8, wherein said antisense nucleic acid molecule has at least 20 nucleotides complementary to SEQ ID NO: 1.

10 10. The method of claim 9, wherein said antisense nucleic acid molecule has at least 20 nucleotides complementary to SEQ ID NO: 3.

11. The method of claim 1, further comprising reducing the expression or activity of L-selectin
15 sulfotransferase-2 (LSST-2) in said subject.

12. An isolated L-selectin antagonist, comprising an extended core 1 structure comprising the oligosaccharide $\text{Gal}\beta 1-4(\text{SO}_3-6)\text{GlcNAc}\beta 1-3\text{Gal}\beta 1-3\text{GalNAc}$.

13. The isolated L-selectin antagonist of
20 claim 12, comprising the oligosaccharide $\text{NeuNAc}\alpha 2-3\text{Gal}\beta 1-4[\text{sulfo-6}(\text{Fuc}\alpha 1-3)\text{GlcNAc}]\beta 1-3\text{Gal}\beta 1-3\text{GalNAc}\alpha 1$.

14. The isolated L-selectin antagonist of claim 12, comprising two or more of the oligosaccharides
25 $\text{Gal}\beta 1-4(\text{SO}_3-6)\text{GlcNAc}\beta 1-3\text{Gal}\beta 1-3\text{GalNAc}$.

15. The isolated L-selectin antagonist of claim 13, comprising two or more of the oligosaccharides NeuNAc α 2-3Gal β 1-4[sulfo-6(Fuc α 1-3)GlcNAc] β 1-3Gal β 1-3GalNAc α 1.

5 16. An isolated nucleic acid molecule, comprising a nucleic acid sequence encoding a L-selectin ligand sulfotransferase (LSST-2) or active fragment thereof, wherein said LSST-2 or active fragment thereof directs expression of a MECA-79 antigen in Chinese
10 hamster ovary (CHO) cells.

17. The isolated nucleic acid molecule of claim 16, wherein said LSST-2 has substantially the amino acid sequence of SEQ ID NO: 6.

15 18. The isolated nucleic acid molecule of claim 17, comprising a nucleic acid sequence encoding SEQ ID NO: 6.

19. The isolated nucleic acid molecule of claim 18, comprising SEQ ID NO: 5.

20 20. An isolated polypeptide, comprising an amino acid sequence encoding a LSST-2 or active fragment thereof, wherein said LSST-2 or active fragment thereof directs expression of a MECA-79 antigen in CHO cells.

21. The isolated polypeptide of claim 20, wherein said LSST-2 has substantially the amino acid
25 sequence of SEQ ID NO: 6.

22. The isolated polypeptide of claim 21,
wherein said LSST-2 has the amino acid sequence SEQ ID
NO: 6.

23. An isolated nucleic acid molecule,
5 comprising a nucleic acid sequence encoding substantially
the amino acid sequence of intestinal GlcNAc
6-sulfotransferase (I-GlcNAc6ST) or an active fragment
thereof.

24. The isolated nucleic acid molecule of
10 claim 23, wherein said I-GlcNAc6ST has substantially the
amino acid sequence of SEQ ID NO: 8.

25. The isolated nucleic acid molecule of
claim 24, comprising a nucleic acid sequence encoding SEQ
ID NO: 8.

15 26. The isolated nucleic acid molecule of
claim 25, comprising SEQ ID NO: 7.

27. An isolated polypeptide, comprising an
amino acid sequence encoding substantially the amino acid
sequence of intestinal GlcNAc 6-sulfotransferase
20 (I-GlcNAc6ST) or an active fragment thereof.

28. The isolated polypeptide of claim 27,
wherein said I-GlcNAc6ST has substantially the amino acid
sequence of SEQ ID NO: 8.

29. The isolated polypeptide of claim 28,
25 wherein said I-GlcNAc6ST has the amino acid sequence SEQ
ID NO: 8.

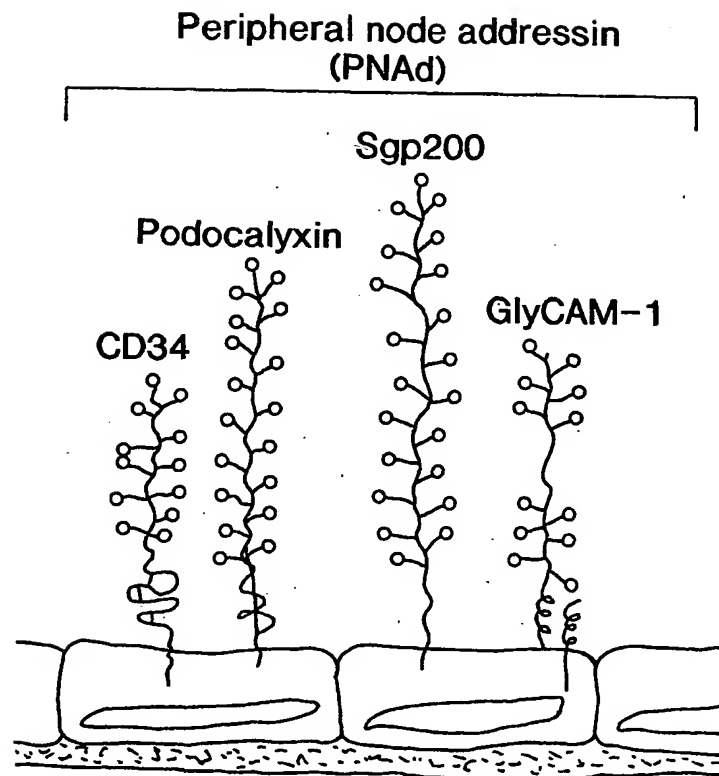


FIG. 1

CTGGCTGGCCAGGATGAAGTATCTCCGGCACCGGCGGCCCAATGCCACCCTCATTCTGGC 60
M K Y L R H R R P N A T L I L A 16

CATCGGCGCTTTCACCCCTCCTCCTCTTCAGTCTGCTAGTGTCAACCACCCACCTGCAAGGT 120
I G A F T L L L F S L L V S P P T C K V 36

CCAGGAGCAGCCACCGGCGATCCCCGAGGCCCTGGCCTGGCCCACTCCACCCACCCGCCC 180
Q E Q P P A I P E A L A W P T P P T R P 56

AGCCCCGGCCCCGTGCCATGCCAACACCTCTATGGTCACCCACCCGGA CTTCGCCACGCA 240
A P A P C H A N T S M V T H P D F A T Q 76

GCCGCAGCACGTTTCAGAACTTCCTCCTGTACAGACACTGCCGCCACTTTCCCTGCTGCA 300
P Q H V Q N F L L Y R H C R H F P L L Q 96

GGACGTGCCCCCTCTAAGTGCGCGCAGCCGGTCTTCCTGCTGCTGGTGATCAAGTCCTC 360
D V P P S K C A Q P V F L L L V I K S S 116

CCCTAGCAACTATGTGCGCCGCGAGCTGCTGCGGCGCACGTGGGGCCGCGAGCGCAAGGT 420
P S N Y V R R E L L R R T W G R E R K V 136

ACGGGGTTTGCAGCTGCGCCTCCTCTTCCTGGTGCGCACAGCCTCCAACCCGCACGAGGC 480
R G L Q L R L L F L V G T A S N P H E A 156

CCGCAAGGTCAACCGGCTGCTGGAGCTGGAGGCACAGACTCACGGAGACATCCTGCAGTG 540
R K V N R L L E L E A Q T H G D I L Q W 156

GGACTTCCACGACTCCTTCTTCAACCTCACGCTCAAGCAGGTCCTGTTCTTACAGTGGCA 600
D F H D S F F N L T L K Q V L F L Q W Q 176

GGAGACAAGGTGCGCCAACGCCAGCTTCGTGCTCAACGGGGATGATGACGTCTTTGCACA 660
E T R C A N A S F V L N G D D D V F A H 196

CACAGACAACATGGTCTTCTACCTGCAGGACCATGACCCTGGCCGCCACCTCTTCGTGGG 720
T D N M V F Y L Q D H D P G R H L F V G 216

GCAACTGATCCAAAACGTGGGCCCCATCCGGGCTTTTTGGAGCAAGTACTATGTGCCAGA 780
Q L I Q N V G P I R A F W S K Y Y V P E 236

GGTGGTGA CT CAGAATGAGCGGTACCCACCCTATTGTGGGGGTGGTGGCTTCTTGCTGTC 840
V V T Q N E R Y P P Y C G G G G F L L S 256

CCGCTTACGGCCGCTGCCCTGCGCCGTGCTGCCCATGTCTTGACATCTTCCCCATTGA 900
R F T A A A L R R A A H V L D I F P I D 276

TGATGTCTTCTGCGGTATGTGTCTGGAGCTTGAGGGACTGAAGCCTGCCTCCCACAGCGG 960
D V F L G M C L E L E G L K P A S H S G 296

CATCCGCACGTCTGGCGTGCGGGCTCCATCGAACACCTGTCTCTCTTGACCCCTGCTT 1020
I R T S G V R A P S Q H L S S F D P C F 316

CTACCGAGACCTGCTGCTGGTGCACCGCTTCTACCTTATGAGATGCTGCTCATGTGGGA 1080
Y R D L L L V H R F L P Y E M L L M W D 336

FIG. 2

SUBSTITUTE SHEET (RULE 26)

TGCGCTGAACCAGCCCAACCTCACCTGCGGCAATCAGACACAGATCTACTGAGTCAGCAT 1140
A L N Q P N L T C G N Q T Q I Y * 352

CAGGCATCCGCACGTCTGGCGTGCGGGCTCCATCGCAACACCTGTCCTCCTTTGACCCCT 1200
GCTTCTAC 1210

FIG. 2 CONT.

AGGCTCCGCCCCACGCCATGCGGCTGCCAAGGCAGAGCCCCTACGAGATCCTCCTCCTG	60
M R L P R Q S P Y E I L L L	14
GTCTTGGTCGCCTTGCTGGTGCTGCTGCTGCTCCTGACCAGCAAGTCACCGCCCAGCTGC	120
V L V A L L V L L L L L T S K S P P S C	34
TCCGCCCCCTGAGAGGTCCAAGGAGCCTGAAGACAACCCCGGGTGGGCCACGGGCCACCCC	180
S A P E R S K E P E D N P G W A T G H P	54
GCCCGGTGCCGAGCTAATCTATCCGTGTCCTCGCACCCCGACTTCGCGGGGCTGCCCTTG	240
A R C R A N L S V S S H P D F A G L P L	74
CACGTGCGCGACTTCTTGTTCTACCGCCACTGCCGCGACTTCCCAGTGCTCCGAGAGCCG	300
H V R D F L F Y R H C R D F P V L R E P	94
CGGGTTACCAAGTGCGCGGAGCCCGTGTTCTGCTGCTCGCCATCAAGTCCTCGCCTGCA	360
R V T K C A E P V F L L L A I K S S P A	114
AACTATGGGCGCCGCCAGATGCTGCGCACGACGTGGGCGCGCGAGAGACGGGTGCGTGGG	420
N Y G R R Q M L R T T W A R E R R V R G	134
GCGCCACTGCGCCGCCTCTTCCTTGTTGGGCTCAGACCGCGACCCACAACAAGCACGCAAA	480
A P L R R L F L V G S D R D P Q Q A R K	154
TACAACCGACTGCTGGAGCTGGAAGCGCAGAAATACGGCGACATTCTCCAGTGGGATTTC	540
Y N R L L E L E A Q K Y G D I L Q W D F	174
CATGACTCCTTCTTTAACCTGACGCTTAAGCAGGTCCTTTTCTGGAGTGGCAGCTAACC	600
H D S F F N L T L K Q V L F L E W Q L T	194
TACTGTACCAACGCCAGCTTCGTGCTCAATGGGGACGACGATGTGTTGCGACACACGGAC	660
Y C T N A S F V L N G D D D V F A H T D	214
AACATGGTCACCTACCTGCAGGACCACGACCCGGACCAACACCTCTTCGTGGGGCACCTG	720
N M V T Y L Q D H D P D Q H L F V G H L	234
ATCCAGAACGTGGGTCCCATCCGGGTGCCCTGGAGCAAGTACTTCATCCCCGCTCTGGTG	780
I Q N V G P I R V P W S K Y F I P A L V	254
ATGGCGGAGGACAGATACCCGCCCTACTGTGGTGGCGGGCGGCTTCCTGCTGTGCGGTTTT	840
M A E D R Y P P Y C G G G G F L L S R F	274
ACCGTGGCCGCCCTACGTGCGCGCGCGCGTCCTCCCCATGTTCCCAATCGACGACGTG	900
T V A A L R R A A R V L P M F P I D D V	294
TTCCTGGGCATGTGTCTGCAGCAGCAGGGTCTGGCTCCCGGGACGCACAGCGGAGTGGCG	960
F L G M C L Q Q Q G L A P G T H S G V R	314
ACTGCGGGGGTTTTCCCCCCTAGCCACGTGTGTCATCCTTCGACCCCTGCTTCTACCGC	1020
T A G V F P P S P R V S S F D P C F Y R	334
GACCTGCTCCTCGTGCACCGCTTCTGCCCCTTCGAGATGCTGCTGATGTGGGATGCGCTG	1080
D L L L V H R F L P F E M L L M W D A L	354

FIG. 3

SUBSTITUTE SHEET (RULE 26)

AACCAGCCCCAGCTCCTCTGCGGCAGGCAGAGCCCCGCCTACTGAGAGGTTTGGGGGAGT	1140
N Q P Q L L C G R Q S P A Y *	368
TGACATCCCCTAGCTCATGTCCTGCCTCATCCACGTGCAAAGGGCTGGCTTCAAGGAGAA	1200
GTTCAAAGTGAGGGGCAGAAAGGTGGGTCTGAGGAGTTCATAGGGCAAACCTCCTAAGTAC	1260
GCTTGGAACCCTCTTGGTACTGTTACAGCAGGAACCTCTGAGTCTAGCCAACCTCTGAGT	1320
GGCTCTAAGTGGCCGCT	1337

FIG. 3 CONT.

1 TTGGCCAGAAGGGGAATAGAAGGCCAAACAATAAAACAGCAGCCCCAACTCCACCCTTTCTG 60
61 TTTGTTTCCTTAAAGGTCTTCCACTTCAGCACAAATGCTACTGCCTAAAAAATGAAGCTCC 120
M K L L
121 TGCTGTTTCTGGTTTCCCAGATGGCCATCTTGGCTCTATTCTTCCACATGTACAGCCACA 180
L F L V S Q M A I L A L F F H M Y S H N
181 ACATCAGCTCCCTGTCTATGAAGGCACAGCCCGAGCGCATGCACGTGCTGGTTCTGTCTT 240
I S S L S M K A Q P E R M H V L V L S S
241 CCTGGCGCTCTGGCTCTTCTTTTGTGGGGCAGCTTTTGGGCAGCACCCAGATGTTTTCT 300
W R S G S S F V G Q L F G Q H P D V F Y
301 ACCTGATGGAGCCCGCCTGGCACGTGTGGATGACCTTCAAGCAGAGCACCGCCTGGATGC 360
L M E P A W H V W M T F K Q S T A W M L
361 TGCACATGGCTGTGCGGGATCTGATACGGGCCGTCTTCTTGTGCGACATGAGCGTCTTTG 420
H M A V R D L I R A V F L C D M S V F D
421 ATGCCTACATGGAACCTGGTCCCCGGAGACAGTCCAGCCTCTTTCAGTGGGAGAACAGCC 480
A Y M E P G P R R Q S S L F Q W E N S R
481 GGGCCCTGTGTTCTGCACCTGCCTGTGACATCATCCACAAGATGAAATCATCCCCGGG 540
A L C S A P A C D I I P Q D E I I P R A
541 CTCACTGCAGGCTCCTGTGCAGTCAACAGCCCTTTGAGGTGGTGGAGAAGGCCTGCCGCT 600
H C R L L C S Q Q P F E V V E K A C R S
601 CCTACAGCCACGTGGTGTCAAGGAGGTGCGCTTCTTCAACCTGCAGTCCCTCTACCCGC 660
Y S H V V L K E V R F F N L Q S L Y P L
661 TGCTGAAAGACCCCTCCCTCAACCTGCATATCGTGCACCTGGTCCGGGACCCCCGGGCCG 720
L K D P S L N L H I V H L V R D P R A V
721 TGTTCCGTTCCCGAGAACGCACAAAGGGAGATCTCATGATTGACAGTCGCATTGTGATGG 780
F R S R E R T K G D L M I D S R I V M G
781 GGCAGCATGAGCAAAAACCTCAAGAAGGAGGACCAACCCTACTATGTGATGCAGGTCATCT 840
Q H E Q K L K K E D Q P Y Y V M Q V I C
841 GCCAAAGCCAGCTGGAGATCTACAAGACCATCCAGTCTTGCCCAAGGCCCTGCAGGAAC 900
Q S Q L E I Y K T I Q S L P K A L Q E R

FIG. 4

901 GCTACCTGCTTGTGCGCTATGAGGACCTGGCTCGAGCCCCTGTGGCCCAGACTTCCCGAA 960
Y L L V R Y E D L A R A P V A Q T S R M

961 TGTATGAATTCGTGGGATTGGAATTCTTGCCCCATCTTCAGACCTGGGTGCATAACATCA 1020
Y E F V G L E F L P H L Q T W V H N I T

1021 CCCGAGGCAAGGGCATGGGTGACCACGCTTTCACACAAATGCCAGGGATGCCCTTAATG 1080
R G K G M G D H A F H T N A R D A L N V

1081 TCTCCCAGGCTTGGCGCTGGTCTTTGCCCTATGAAAAGGTTTCTCGACTTCAGAAAGCCT 1140
S Q A W R W S L P Y E K V S R L Q K A C

1141 GTGGCGATGCCATGAATTTGCTGGGCTACCGCCACGTCAGATCTGAACAAGAACAGAGAA 1200
G D A M N L L G Y R H V R S E Q E Q R N

1201 ACCTGTTGCTGGATCTTCTGTCTACCTGGACTGTCCCTGAGCAAATCCACTAAGAGGGTT 1260
L L L D L L S T W T V P E Q I H *

1261 GAGAAGGCTTTGCTGCCACCTGGTGTGAGCCTCAGTCACTTTCTCTGAATGCTTCTGAGC 1320

1321 CTTGCCTACATCT 1333

FIG. 4 CONT.


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30GaIT-I : -----HASKVSCLYLSVVCASALWYL-----20      40      60      80      100      120      140      160      180      200      220      240      260      280      300      320      340      360      380      400      420      440      460      480
30GaIT-II : -----MLQWRRRHCCFAKWTSPKRSLLRTPLTGLVSLVFLFAFLFFNHQWLCGRPGKFNKPVYTYFRGFRSTKSETHSSLRITWKEVAPQTLRPHIASNS : 31
30GaIT-III : -----HAPAVLTALPNRHSLSRLKWSLLLSLSFL-----SITRPTS-----S : 100
30GaIT-IV : -----HPLSLFRVLLAVLLLVIIWTLFG-----VWYLSLP----- : 39
30GnT : -----LWGGFFSGR-----PGLG----- : 29
30GnT : -----HVFRR----- : 6

30GaIT-I : -----120      140      160      180      200      220      240      260      280      300      320      340      360      380      400      420      440      460      480
30GaIT-II : -----FSLHTVARKNFTFGHIR-TRPINPHSFEFLINEPNKCEKNIP-----FVITISTTHKEFDAPQAIREDINSGOENNFRQ--IKIATLFLGKNA : 122
30GaIT-III : -----NTELSPOQVTLQNTLSANGSIYNEKGTGHPNSYHFKYTIINEPEKCEKSP-----FLITLAAEPGQIEARAIQITINGNETLAPQ-IQIRVFLGISI : 195
30GaIT-IV : -----HYNVIERYVMWMPYFY--EPIYRQDFRFTLREHNSCHQIP-----FLVILVTSRPSDVKAPQAIQITINGEKKSWAG-YEVLTFELGQOA : 122
30GnT : -----EELLSSLASLLPAPASPGPLALPRLLISNSH-ACGSGGPPP--FLILVCTAPEHLNQNINAKISWGAIREANG-FRVQTLFLGKPR : 115
30GnT : -----AMRHRAVALGLGLAFPGT--TLLYLARCASEGETPSASGAARPRAKAFLANVASAPRAVERITAVRSIDMLAPERRGQPEDVWARFALGTGG : 96

30GaIT-I : -----220      240      260      280      300      320      340      360      380      400      420      440      460      480
30GaIT-II : -----VNLQWVEQISQIFHO--ITVEDEFIDSYHQLITLTKTGKGRMATFCSKAKVVMRTDSIIFVANDNATYKILKPKSTKPRRR----- : 201
30GaIT-III : -----KLANG-YLQHAQEEISQRYHO--ITQQEYLDTIVYVILITITLTKTGKGRMATFCSKAKVVMRTDSIIFVANDNATYKILKPKSTKPRRR----- : 276
30GaIT-IV : -----EREDKTLALSIEDCHVLYGO--ITRQDFLITVYVILITITLTKTGKGRMATFCSKAKVVMRTDSIIFVANDNATYKILKPKSTKPRRR----- : 202
30GnT : -----RQ--QLADSSSAHRO--ITQASFOOSYVRLITLTKTGKGRMATFCSKAKVVMRTDSIIFVANDNATYKILKPKSTKPRRR----- : 210
30GnT : -----LGS--EERRALELEQAQHQDILLPLALPDQAVENITATYVLAULTIIOERY-DFEVILKADQDSFARLDALITVQIRAREPARRR----- : 176

30GaIT-I : -----320      340      360      380      400      420      440      460      480
30GaIT-II : -----YFTGVYING-GPIFOVRSKMYMPRODLYPOSN--YPPFCSGTGYTIFSAADVAELTYKTSILHTRLLHLLEDVYGLCLRLKLGHPFQNSG-----FNHW : 288
30GaIT-III : -----YFTGYLARGYAPNKNDSKMYHPDOLYPSER--YPPVPCSGTGYTIFSAADVAELTYKTSILHTRLLHLLEDVYGLCLRLKLGHPFQNSG-----FNHW : 367
30GaIT-IV : -----FFTGYPLIDNYSYRGFFHKNHSYQEPFKV--FPPVPCSGTGYTIFSAADVAELTYKTSILHTRLLHLLEDVYGLCLRLKLGHPFQNSG-----FNHW : 293
30GnT : -----RQAVPLLLGLVAMRVRPTRTPESRHHVSEELPEHNGRPPLPPASGTGVLISAVQLILKVASRAPPLPLEDFVVCVSARRGGLAPTHCVKLAGATHY : 310
30GnT : -----LWGGFFSGR--GIVKPG-GRWREAAQLCD-YVLPVIALGCGVLSADLVHYVIRLSREYLRAMHSEIDVSLGTLVAPVQVQREHDP--FDTE : 261

30GaIT-I : -----420      440      460      480
30GaIT-II : -----KNAYSILGRYRVITYQISPEEYHRIWDCSSKK--H--LRC----- : 326
30GaIT-III : -----RVSYSSGKYSHCHITSQFQPSSEIKYWNHQQNK--H--NACANAAKEKAGRYRHRKLH----- : 422
30GaIT-IV : -----RTHLDVYQLRRVLAAGFFSKELITFWQILRN--TTCHY----- : 331
30GnT : -----PLDR-CGYGKFLITSIKVDPWQIQEAMKLVSGNANGERTA--PFCSMLOG-FLGTLRCRFTIAWFSS----- : 371
30GnT : -----YKSR-GANNQYLT-UKQSPEDULEKQVULHEGRLCXHEVQLRLSYVDWSAPPSCCQCKEGVP----- : 325

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FIG. 5

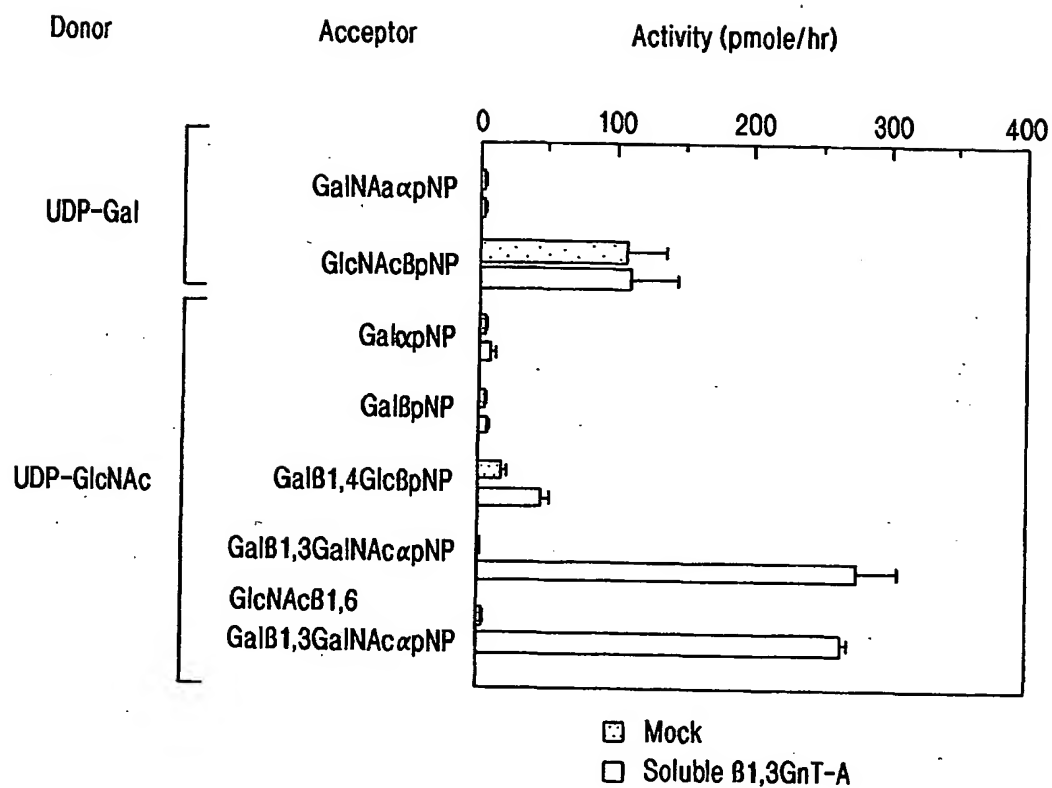


FIG. 6

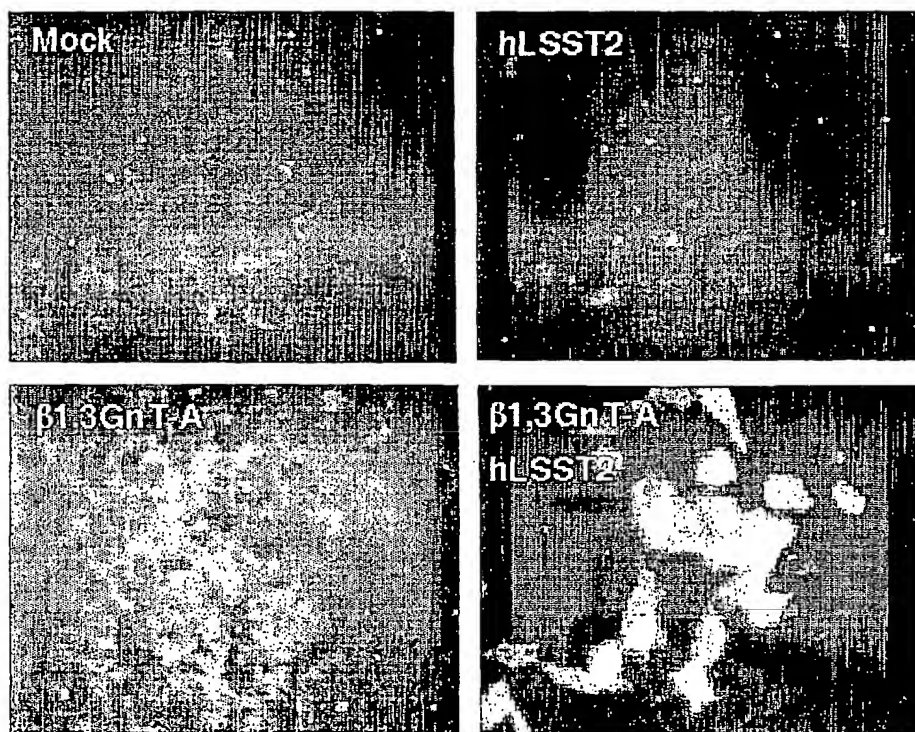


FIG. 7

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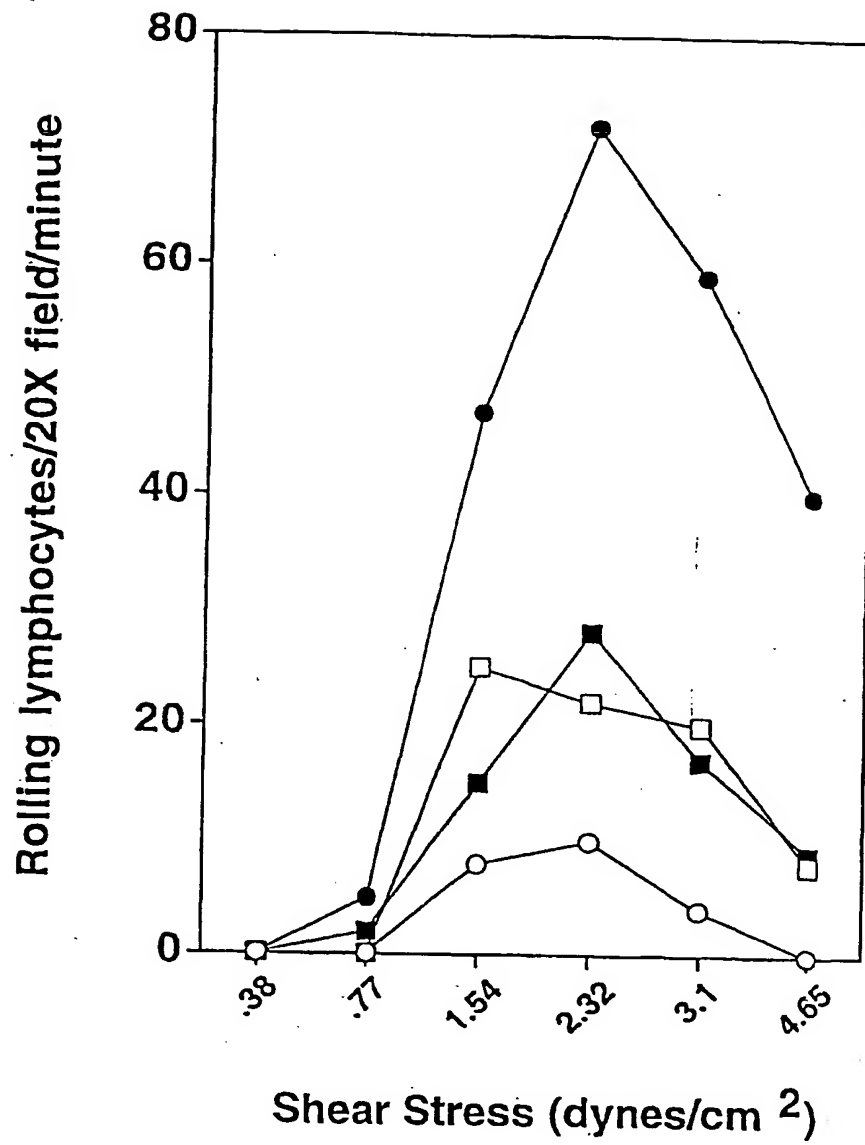


FIG. 8

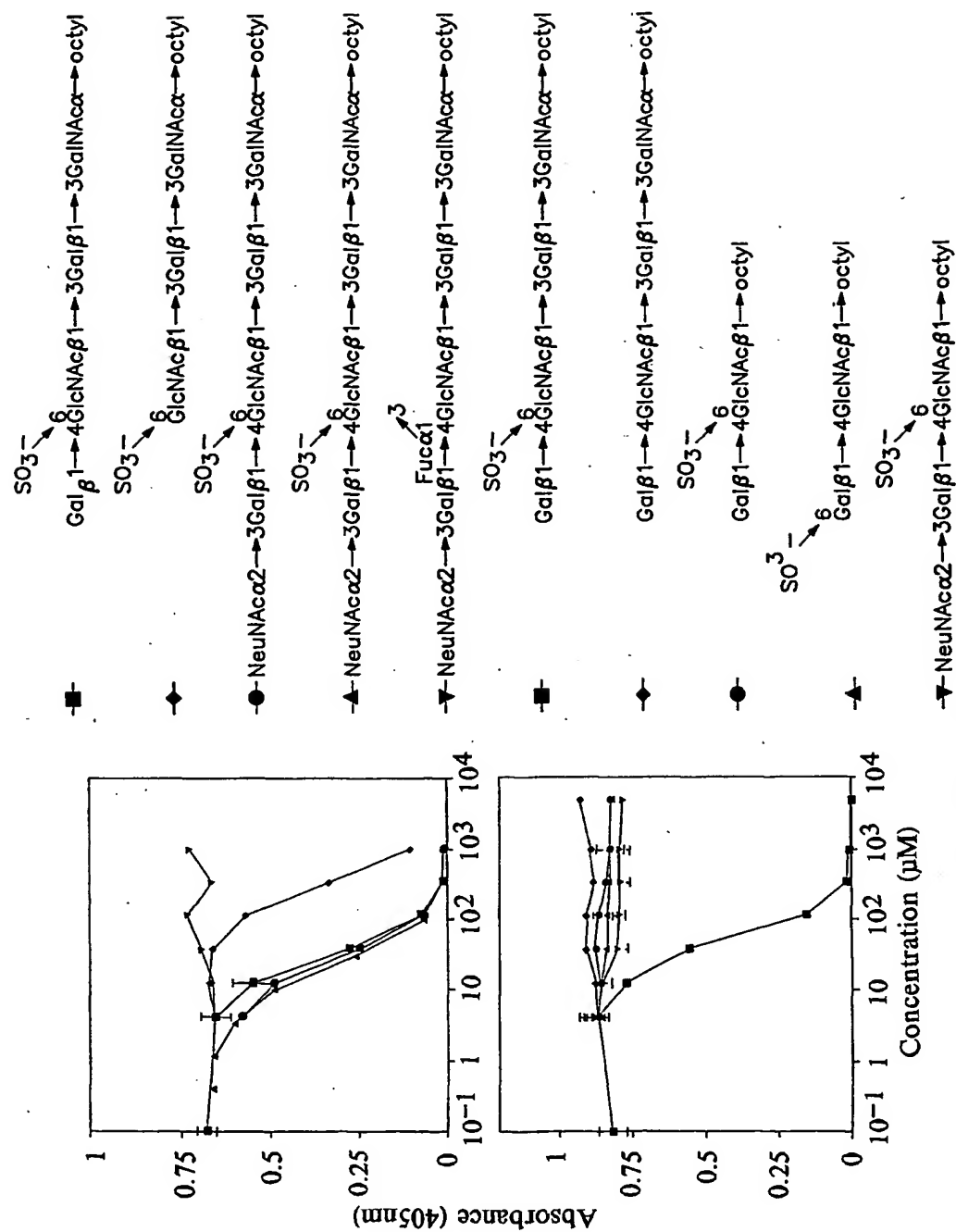


FIG. 9

TGAGCGGCTCTTTGTGTGCGCCCTGGGTGCGCAGCGCAGAAGCGCAGCGGGCAGCGCAGG 60
CCCTAGCCAGAGGTATGCGGCTACCCCGTTTCTCCAGCACTGTCATGCTTTTCGCTCCTGA 120
M R L P R F S S T V M L S L L M
TGGTACAGACTGGCATCCTGGTCTTCCTGGTCTCCCGGCAAGTGCCATCGTCCCCAGCAG 180
V Q T G I L V F L V S R Q V P S S P A G
GCCTTGGGGAGCGTGTGCACGTGCTGGTACTGTCCTCGTGGCGCTCGGGCTCGTCTTCG 240
L G E R V H V L V L S S W R S G S S F V
TGGGCCAGCTCTTCAGCCAACACCCCGATGTCTTCTACCTGATGGAGCCGGCTTGGCAGC 300
G Q L F S Q H P D V F Y L M E P A W H V
TCTGGGATACGTTGTGCGAGGGCAGTGCCCCGCACTCCACATGGCCGTGCGTGACCTGA 360
W D T L S Q G S A P A L H M A V R D L I
TCCGCTCAGTGTTCTTATGCGACATGGACGTATTTGATGCCTACCTGCCCTGGCGCCGCA 420
R S V F L C D M D V F D A Y L P W R R N
ACATCTCGGATCTCTTCCAGTGGGCGGTGAGCCGCGCATTGTGCTCACCTCCGGTCTGCG 480
I S D L F Q W A V S R A L C S P P V C E
AAGCCTTCGCTCGTGGCAACATCAGCAGCGAGGAGGTGTGTAAGCCTCTGTGCGCAACGC 540
A F A R G N I S S E E V C K P L C A T R
GGCCCTTCGGCCTGGCTCAGGAAGCCTGCAGCTCCTATAGTCACGTGCTGCTCAAGGAGG 600
P F G L A Q E A C S S Y S H V V L K E V
TGCGCTTCTTTAACCTACAGGTGCTCTACCCGCTGCTCAGCGACCCTGCGCTCAACCTGC 660
R F F N L Q V L Y P L L S D P A L N L R
GCATCGTGACCTAGTGCGCGACCCGCGGGCCGTGCTGCGCTCCCGAGAGCAGACAGCCA 720
I V H L V R D P R A V L R S R E Q T A K
AGGCGCTGGCAGGGGACAATGGCATCGTCTGGGTACCAACGGCACGTGGGTGGAGGCGG 780
A L A R D N G I V L G T N G T W V E A D
ACCCCGGCTGCGCGTGGTCAACGAGGTATGCCGCAGCCATGTGCGCATCGCAGAGGCAG 840
P R L R V V N E V C R S H V R I A E A A
CCTTGACAAGCCGCCGCCCTTCTTGCAAGATCGCTACCGCCTGGTGGCTACGAGGATC 900
L H K P P P F L Q D R Y R L V R Y E D L
TGGCCCCGGGACCCACTCACCGTAATCCGTGAACCTCTATGCCTTCACCGGCCTGGGTCTCA 960
A R D P L T V I R E L Y A F T G L G L T

FIG. 10

CGCCGCAGCTCCAGACTTGGATCCACAATATCACGCATGGTTCAGGGCCAGGCGCGCGCC 1020
P Q L Q T W I H N I T H G S G P G A R R

GTGAAGCCTTCAAGACCACATCCAGGGATGCGCTCAGTGTATCCCAGGCCTGGCGCCACA 1080
E A F K T T S R D A L S V S Q A W R H T

CGCTGCCCTTTGCCAAGATTGCGCGGGTCCAGGAACGTGCGGGGGTGCAGTGCAGCTGC 1140
L P F A K I R R V Q E L C G G A L Q L L

TGGGTTACCGGTCTGTGCATTTCGGAGCTTGAGCAAAGGGACCTCTCTCTGGACCTCCTGC 1200
G Y R S V H S E L E Q R D L S L D L L L

TGCCAAGAGGCATGGACAGTTTCAAGTGGGCATCGTCCACGGAGAAGCAACCGGAATCTT 1260
P R G M D S F K W A S S T E K Q P E S *

AGAATTTTAGTGGAGAGACCCAGCTATAACATTAGGGTCTATTGGAGTATGATAAAGAAG 1320

GGGCTTGGAGAACCCAAAAGCAAGTAGCTGGGAGTGTGAGTGATCTTGTCTGTACTAGG 1380

AAAGGATGGAGTCCAAATCCCACATCTCTTTCTGTCCAGATTGTAGTTTTTCGGTTTTGGT 1440

CTTTTAGGGTTTGGATTCCCACCAAGTACTATCGAATGGAAAGCAAAAGCTGTGCCCACT 1500

TCCTTCAGAGAGGCAGCCAGCCTCCTACTAAAGCACTTCCTTTCTCGTTGACTCTCTCCC 1560

CTCTTTGATCATACCATGCAATCGCAGAGAATGGGGTCCCAGGCCTGCTCTGGAGTGCGG 1620

GAAAGGCGCGGCTGTGGGCTGGCTCCTAAAATCTGTGCACCTGCCTCTCGTTGGCTCACC 1680

CAGACCTCTGCTCACTGCCACGCCCTAGTATCTCAGTCCATCATAGACTTGGACAGTTAT 1740

GGGCCTGGTCAAGGAGGAAAATGAGACGATGCTTCCCTCTGTGATTCTCTGCCTGACCTT 1800

CTAGAAGGGAATCCAGGCACACACAACCATACCTGAGGAGGATGGCTTTTTTAATGAAT 1860

CTTTGATTTGTCCTGAGATGAAAGATCCTAATTTATGGAAATAAACATAAATATGCTGCG 1920

TGATCCAAAAAAAAA 1937

FIG. 10 CONT.

SEQUENCE LISTING

<110> The Burnham Institute
Fukuda, Minoru
Yeh, Jiunn-Chern
Hiraoka, Nobuyoshi

<120> Identification of the Meca-79 Antigen
and Related Methods of Treating L-Selectin-Mediated
Conditions

<130> FP-LJ 4631

<140> PCT/US01/15452

<141> 2001-05-10

<150> US 09/569,320

<151> 2000-05-11

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      15              20              25

gtg tca cca ccc acc tgc aag gtc cag gag cag cca ccg gcg atc ccc      145
Val Ser Pro Pro Thr Cys Lys Val Gln Glu Gln Pro Pro Ala Ile Pro
      30              35              40

gag gcc ctg gcc tgg ccc act cca ccc acc cgc cca gcc ccg gcc ccg      193
Glu Ala Leu Ala Trp Pro Thr Pro Pro Thr Arg Pro Ala Pro Ala Pro
      45              50              55              60

tgc cat gcc aac acc tct atg gtc acc cac ccg gac ttc gcc acg cag      241
Cys His Ala Asn Thr Ser Met Val Thr His Pro Asp Phe Ala Thr Gln
          65              70              75

ccg cag cac gtt cag aac ttc ctc ctg tac aga cac tgc cgc cac ttt      289

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Pro	Gln	His	Val	Gln	Asn	Phe	Leu	Leu	Tyr	Arg	His	Cys	Arg	His	Phe		
			80					85					90				
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Pro	Leu	Leu	Gln	Asp	Val	Pro	Pro	Ser	Lys	Cys	Ala	Gln	Pro	Val	Phe		
			95				100					105					
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Leu	Leu	Leu	Val	Ile	Lys	Ser	Ser	Pro	Ser	Asn	Tyr	Val	Arg	Arg	Glu		
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Leu	Leu	Arg	Arg	Thr	Trp	Gly	Arg	Glu	Arg	Lys	Val	Arg	Gly	Leu	Gln		
125							130				135				140		
ctg	cgc	ctc	ctc	ttc	ctg	gtg	ggc	aca	gcc	tcc	aac	ccg	cac	gag	gcc	481	
Leu	Arg	Leu	Leu	Phe	Leu	Val	Gly	Thr	Ala	Ser	Asn	Pro	His	Glu	Ala		
				145					150					155			
cgc	aag	gtc	aac	cgg	ctg	ctg	gag	ctg	gag	gca	cag	act	cac	gga	gac	529	
Arg	Lys	Val	Asn	Arg	Leu	Leu	Glu	Leu	Glu	Ala	Gln	Thr	His	Gly	Asp		
			160					165					170				
atc	ctg	cag	tgg	gac	ttc	cac	gac	tcc	ttc	ttc	aac	ctc	acg	ctc	aag	577	
Ile	Leu	Gln	Trp	Asp	Phe	His	Asp	Ser	Phe	Phe	Asn	Leu	Thr	Leu	Lys		
			175				180						185				
cag	gtc	ctg	ttc	tta	cag	tgg	cag	gag	aca	agg	tgc	gcc	aac	gcc	agc	625	
Gln	Val	Leu	Phe	Leu	Gln	Trp	Gln	Glu	Thr	Arg	Cys	Ala	Asn	Ala	Ser		
			190				195					200					
ttc	gtg	ctc	aac	ggg	gat	gat	gac	gtc	ttt	gca	cac	aca	gac	aac	atg	673	
Phe	Val	Leu	Asn	Gly	Asp	Asp	Asp	Val	Phe	Ala	His	Thr	Asp	Asn	Met		
205							210				215				220		
gtc	ttc	tac	ctg	cag	gac	cat	gac	cct	ggc	cgc	cac	ctc	ttc	gtg	ggg	721	
Val	Phe	Tyr	Leu	Gln	Asp	His	Asp	Pro	Gly	Arg	His	Leu	Phe	Val	Gly		
				225					230					235			
caa	ctg	atc	caa	aac	gtg	ggc	ccc	atc	cgg	gct	ttt	tgg	agc	aag	tac	769	
Gln	Leu	Ile	Gln	Asn	Val	Gly	Pro	Ile	Arg	Ala	Phe	Trp	Ser	Lys	Tyr		
			240					245					250				
tat	gtg	cca	gag	gtg	gtg	act	cag	aat	gag	cgg	tac	cca	ccc	tat	tgt	817	
Tyr	Val	Pro	Glu	Val	Val	Thr	Gln	Asn	Glu	Arg	Tyr	Pro	Pro	Tyr	Cys		
			255				260					265					
ggg	ggg	ggg	ggc	ttc	ttg	ctg	tcc	cgc	ttc	acg	gcc	gct	gcc	ctg	cgc	865	
Gly	Gly	Gly	Gly	Phe	Leu	Leu	Ser	Arg	Phe	Thr	Ala	Ala	Ala	Leu	Arg		
			270				275				280						
cgt	gct	gcc	cat	gtc	ttg	gac	atc	ttc	ccc	att	gat	gat	gtc	ttc	ctg	913	
Arg	Ala	Ala	His	Val	Leu	Asp	Ile	Phe	Pro	Ile	Asp	Asp	Val	Phe	Leu		
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PCT/US01/15452

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<212> PRT
<213> Homo Sapien
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			20					25					30		
Thr	Cys	Lys	Val	Gln	Glu	Gln	Pro	Pro	Ala	Ile	Pro	Glu	Ala	Leu	Ala
		35					40					45			
Trp	Pro	Thr	Pro	Pro	Thr	Arg	Pro	Ala	Pro	Ala	Pro	Cys	His	Ala	Asn
	50					55					60				
Thr	Ser	Met	Val	Thr	His	Pro	Asp	Phe	Ala	Thr	Gln	Pro	Gln	His	Val
65					70					75					80
Gln	Asn	Phe	Leu	Leu	Tyr	Arg	His	Cys	Arg	His	Phe	Pro	Leu	Leu	Gln
			85						90					95	
Asp	Val	Pro	Pro	Ser	Lys	Cys	Ala	Gln	Pro	Val	Phe	Leu	Leu	Leu	Val
			100					105					110		
Ile	Lys	Ser	Ser	Pro	Ser	Asn	Tyr	Val	Arg	Arg	Glu	Leu	Leu	Arg	Arg
		115					120					125			
Thr	Trp	Gly	Arg	Glu	Arg	Lys	Val	Arg	Gly	Leu	Gln	Leu	Arg	Leu	Leu
		130				135					140				
Phe	Leu	Val	Gly	Thr	Ala	Ser	Asn	Pro	His	Glu	Ala	Arg	Lys	Val	Asn
145					150					155					160
Arg	Leu	Leu	Glu	Leu	Glu	Ala	Gln	Thr	His	Gly	Asp	Ile	Leu	Gln	Trp
			165						170					175	
Asp	Phe	His	Asp	Ser	Phe	Phe	Asn	Leu	Thr	Leu	Lys	Gln	Val	Leu	Phe
			180					185					190		
Leu	Gln	Trp	Gln	Glu	Thr	Arg	Cys	Ala	Asn	Ala	Ser	Phe	Val	Leu	Asn
		195					200					205			
Gly	Asp	Asp	Asp	Val	Phe	Ala	His	Thr	Asp	Asn	Met	Val	Phe	Tyr	Leu

210 215 220
 Gln Asp His Asp Pro Gly Arg His Leu Phe Val Gly Gln Leu Ile Gln
 225 230 235 240
 Asn Val Gly Pro Ile Arg Ala Phe Trp Ser Lys Tyr Tyr Val Pro Glu
 245 250 255
 Val Val Thr Gln Asn Glu Arg Tyr Pro Pro Tyr Cys Gly Gly Gly Gly
 260 265 270
 Phe Leu Leu Ser Arg Phe Thr Ala Ala Ala Leu Arg Arg Ala Ala His
 275 280 285
 Val Leu Asp Ile Phe Pro Ile Asp Asp Val Phe Leu Gly Met Cys Leu
 290 295 300
 Glu Leu Glu Gly Leu Lys Pro Ala Ser His Ser Gly Ile Arg Thr Ser
 305 310 315 320
 Gly Val Arg Ala Pro Ser Gln His Leu Ser Ser Phe Asp Pro Cys Phe
 325 330 335
 Tyr Arg Asp Leu Leu Leu Val His Arg Phe Leu Pro Tyr Glu Met Leu
 340 345 350
 Leu Met Trp Asp Ala Leu Asn Gln Pro Asn Leu Thr Cys Gly Asn Gln
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 Thr Gln Ile Tyr
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 ctc ctc ctg gtc ttg gtc gcc ttg ctg gtg ctg ctg ctg ctc ctg acc 99
 Leu Leu Leu Val Leu Val Ala Leu Leu Val Leu Leu Leu Leu Leu Thr
 15 20 25

 agc aag tca ccg ccc agc tgc tcc gcc cct gag agg tcc aag gag cct 147
 Ser Lys Ser Pro Pro Ser Cys Ser Ala Pro Glu Arg Ser Lys Glu Pro
 30 35 40

 gaa gac aac ccc ggg tgg gcc acg ggc cac ccc gcc cgg tgc cga gct 195
 Glu Asp Asn Pro Gly Trp Ala Thr Gly His Pro Ala Arg Cys Arg Ala
 45 50 55

 aat cta tcc gtg tcc tcg cac ccc gac ttc gcg ggg ctg ccc ttg cac 243
 Asn Leu Ser Val Ser Ser His Pro Asp Phe Ala Gly Leu Pro Leu His
 60 65 70 75

 gtg cgc gac ttc ttg ttc tac cgc cac tgc cgc gac ttc cca gtg ctc 291
 Val Arg Asp Phe Leu Phe Tyr Arg His Cys Arg Asp Phe Pro Val Leu

	80	85	90	
cga gag ccg cgg gtt acc aag tgc gcg gag ccc gtg ttc ctg ctg ctc				339
Arg Glu Pro Arg Val Thr Lys Cys Ala Glu Pro Val Phe Leu Leu Leu				
	95	100	105	
gcc atc aag tcc tcg cct gca aac tat ggg cgc cgc cag atg ctg cgc				387
Ala Ile Lys Ser Ser Pro Ala Asn Tyr Gly Arg Arg Gln Met Leu Arg				
	110	115	120	
acg acg tgg gcg cgc gag aga cgg gtg cgt ggg gcg cca ctg cgc cgc				435
Thr Thr Trp Ala Arg Glu Arg Arg Val Arg Gly Ala Pro Leu Arg Arg				
	125	130	135	
ctc ttc ctt gtg ggc tca gac cgc gac cca caa caa gca cgc aaa tac				483
Leu Phe Leu Val Gly Ser Asp Arg Asp Pro Gln Gln Ala Arg Lys Tyr				
	140	145	150	155
aac cga ctg ctg gag ctg gaa gcg cag aaa tac ggc gac att ctc cag				531
Asn Arg Leu Leu Glu Leu Glu Ala Gln Lys Tyr Gly Asp Ile Leu Gln				
	160	165	170	
tgg gat ttc cat gac tcc ttc ttt aac ctg acg ctt aag cag gtc ctt				579
Trp Asp Phe His Asp Ser Phe Phe Asn Leu Thr Leu Lys Gln Val Leu				
	175	180	185	
ttc ctg gag tgg cag cta acc tac tgt acc aac gcc agc ttc gtg ctc				627
Phe Leu Glu Trp Gln Leu Thr Tyr Cys Thr Asn Ala Ser Phe Val Leu				
	190	195	200	
aat ggg gac gac gat gtg ttc gca cac acg gac aac atg gtc acc tac				675
Asn Gly Asp Asp Asp Val Phe Ala His Thr Asp Asn Met Val Thr Tyr				
	205	210	215	
ctg cag gac cac gac ccg gac caa cac ctc ttc gtg ggg cac ctg atc				723
Leu Gln Asp His Asp Pro Asp Gln His Leu Phe Val Gly His Leu Ile				
	220	225	230	235
cag aac gtg ggt ccc atc cgg gtg ccc tgg agc aag tac ttc atc ccc				771
Gln Asn Val Gly Pro Ile Arg Val Pro Trp Ser Lys Tyr Phe Ile Pro				
	240	245	250	
gct ctg gtg atg gcg gag gac aga tac ccg ccc tac tgt ggt ggc ggc				819
Ala Leu Val Met Ala Glu Asp Arg Tyr Pro Pro Tyr Cys Gly Gly Gly				
	255	260	265	
ggc ttc ctg ctg tcg cgt ttt acc gtg gcc gcc cta cgt cgc gcc gcg				867
Gly Phe Leu Leu Ser Arg Phe Thr Val Ala Ala Leu Arg Arg Ala Ala				
	270	275	280	
cgc gtc ctc ccc atg ttc cca atc gac gac gtg ttc ctg ggc atg tgt				915
Arg Val Leu Pro Met Phe Pro Ile Asp Asp Val Phe Leu Gly Met Cys				
	285	290	295	
ctg cag cag cag ggt ctg gct ccc ggg acg cac agc gga gtg cgc act				963

Leu Gln Gln Gln Gly Leu Ala Pro Gly Thr His Ser Gly Val Arg Thr
 300 305 310 315

 gcg ggg gtt ttc ccc cct agc cca cgt gtg tca tcc ttc gac ccc tgc 1011
 Ala Gly Val Phe Pro Pro Ser Pro Arg Val Ser Ser Phe Asp Pro Cys
 320 325 330

 ttc tac cgc gac ctg ctc ctc gtg cac cgc ttc ctg ccc ttc gag atg 1059
 Phe Tyr Arg Asp Leu Leu Leu Val His Arg Phe Leu Pro Phe Glu Met
 335 340 345

 ctg ctg atg tgg gat gcg ctg aac cag ccc cag ctc ctc tgc ggc agg 1107
 Leu Leu Met Trp Asp Ala Leu Asn Gln Pro Gln Leu Leu Cys Gly Arg
 350 355 360

 cag agc ccc gcc tac tgagaggttt gggggagttg acatccccta gctcatgtcc 1162
 Gln Ser Pro Ala Tyr
 365

tgctcatcc acgtgcaaag ggctggcttc aaggagaagt tcaaagtgag gggcagaaag 1222
 gtgggtctga ggagttcata gggcaaactc ctaagtacgc ttggaaaccc tcttggtact 1282
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 <213> Mus musculus

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 Ser Cys Ser Ala Pro Glu Arg Ser Lys Glu Pro Glu Asp Asn Pro Gly
 35 40 45
 Trp Ala Thr Gly His Pro Ala Arg Cys Arg Ala Asn Leu Ser Val Ser
 50 55 60
 Ser His Pro Asp Phe Ala Gly Leu Pro Leu His Val Arg Asp Phe Leu
 65 70 75 80
 Phe Tyr Arg His Cys Arg Asp Phe Pro Val Leu Arg Glu Pro Arg Val
 85 90 95
 Thr Lys Cys Ala Glu Pro Val Phe Leu Leu Leu Ala Ile Lys Ser Ser
 100 105 110
 Pro Ala Asn Tyr Gly Arg Arg Gln Met Leu Arg Thr Thr Trp Ala Arg
 115 120 125
 Glu Arg Arg Val Arg Gly Ala Pro Leu Arg Arg Leu Phe Leu Val Gly
 130 135 140
 Ser Asp Arg Asp Pro Gln Gln Ala Arg Lys Tyr Asn Arg Leu Leu Glu
 145 150 155 160
 Leu Glu Ala Gln Lys Tyr Gly Asp Ile Leu Gln Trp Asp Phe His Asp
 165 170 175
 Ser Phe Phe Asn Leu Thr Leu Lys Gln Val Leu Phe Leu Glu Trp Gln
 180 185 190
 Leu Thr Tyr Cys Thr Asn Ala Ser Phe Val Leu Asn Gly Asp Asp Asp
 195 200 205

Val Phe Ala His Thr Asp Asn Met Val Thr Tyr Leu Gln Asp His Asp
 210 215 220
 Pro Asp Gln His Leu Phe Val Gly His Leu Ile Gln Asn Val Gly Pro
 225 230 235 240
 Ile Arg Val Pro Trp Ser Lys Tyr Phe Ile Pro Ala Leu Val Met Ala
 245 250 255
 Glu Asp Arg Tyr Pro Pro Tyr Cys Gly Gly Gly Gly Phe Leu Leu Ser
 260 265 270
 Arg Phe Thr Val Ala Ala Leu Arg Arg Ala Ala Arg Val Leu Pro Met
 275 280 285
 Phe Pro Ile Asp Asp Val Phe Leu Gly Met Cys Leu Gln Gln Gln Gly
 290 295 300
 Leu Ala Pro Gly Thr His Ser Gly Val Arg Thr Ala Gly Val Phe Pro
 305 310 315 320
 Pro Ser Pro Arg Val Ser Ser Phe Asp Pro Cys Phe Tyr Arg Asp Leu
 325 330 335
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 340 345 350
 Ala Leu Asn Gln Pro Gln Leu Leu Cys Gly Arg Gln Ser Pro Ala Tyr
 355 360 365

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<220>
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 Met Lys
 1

ctc ctg ctg ttt ctg gtt tcc cag atg gcc atc ttg gct cta ttc ttc 164
 Leu Leu Leu Phe Leu Val Ser Gln Met Ala Ile Leu Ala Leu Phe Phe
 5 10 15

cac atg tac agc cac aac atc agc tcc ctg tct atg aag gca cag ccc 212
 His Met Tyr Ser His Asn Ile Ser Ser Leu Ser Met Lys Ala Gln Pro
 20 25 30

gag cgc atg cac gtg ctg gtt ctg tct tcc tgg cgc tct ggc tct tct 260
 Glu Arg Met His Val Leu Val Leu Ser Ser Trp Arg Ser Gly Ser Ser
 35 40 45 50

ttt gtg ggg cag ctt ttt ggg cag cac cca gat gtt ttc tac ctg atg 308
 Phe Val Gly Gln Leu Phe Gly Gln His Pro Asp Val Phe Tyr Leu Met
 55 60 65

gag ccc gcc tgg cac gtg tgg atg acc ttc aag cag agc acc gcc tgg 356
 Glu Pro Ala Trp His Val Trp Met Thr Phe Lys Gln Ser Thr Ala Trp

70										75										80										
atg	ctg	cac	atg	gct	gtg	cgg	gat	ctg	ata	cgg	gcc	gtc	ttc	ttg	tgc	404														
Met	Leu	His	Met	Ala	Val	Arg	Asp	Leu	Ile	Arg	Ala	Val	Phe	Leu	Cys															
85					90					95																				
gac	atg	agc	gtc	ttt	gat	gcc	tac	atg	gaa	cct	ggt	ccc	cgg	aga	cag	452														
Asp	Met	Ser	Val	Phe	Asp	Ala	Tyr	Met	Glu	Pro	Gly	Pro	Arg	Arg	Gln															
100				105				110																						
tcc	agc	ctc	ttt	cag	tgg	gag	aac	agc	cgg	gcc	ctg	tgt	tct	gca	cct	500														
Ser	Ser	Leu	Phe	Gln	Trp	Glu	Asn	Ser	Arg	Ala	Leu	Cys	Ser	Ala	Pro															
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gcc	tgt	gac	atc	atc	cca	caa	gat	gaa	atc	atc	ccc	cgg	gct	cac	tgc	548														
Ala	Cys	Asp	Ile	Ile	Pro	Gln	Asp	Glu	Ile	Ile	Pro	Arg	Ala	His	Cys															
135					140					145																				
agg	ctc	ctg	tgc	agt	caa	cag	ccc	ttt	gag	gtg	gtg	gag	aag	gcc	tgc	596														
Arg	Leu	Leu	Cys	Ser	Gln	Gln	Pro	Phe	Glu	Val	Val	Glu	Lys	Ala	Cys															
150				155				160																						
cgc	tcc	tac	agc	cac	gtg	gtg	ctc	aag	gag	gtg	cgc	ttc	ttc	aac	ctg	644														
Arg	Ser	Tyr	Ser	His	Val	Val	Leu	Lys	Glu	Val	Arg	Phe	Phe	Asn	Leu															
165				170				175																						
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Gln	Ser	Leu	Tyr	Pro	Leu	Leu	Lys	Asp	Pro	Ser	Leu	Asn	Leu	His	Ile															
180			185				190																							
gtg	cac	ctg	gtc	cgg	gac	ccc	cgg	gcc	gtg	ttc	cgt	tcc	cga	gaa	cgc	740														
Val	His	Leu	Val	Arg	Asp	Pro	Arg	Ala	Val	Phe	Arg	Ser	Arg	Glu	Arg															
195		200				205				210																				
aca	aag	gga	gat	ctc	atg	att	gac	agt	cgc	att	gtg	atg	ggg	cag	cat	788														
Thr	Lys	Gly	Asp	Leu	Met	Ile	Asp	Ser	Arg	Ile	Val	Met	Gly	Gln	His															
215					220					225																				
gag	caa	aaa	ctc	aag	aag	gag	gac	caa	ccc	tac	tat	gtg	atg	cag	gtc	836														
Glu	Gln	Lys	Leu	Lys	Lys	Glu	Asp	Gln	Pro	Tyr	Tyr	Val	Met	Gln	Val															
230				235				240																						
atc	tgc	caa	agc	cag	ctg	gag	atc	tac	aag	acc	atc	cag	tcc	ttg	ccc	884														
Ile	Cys	Gln	Ser	Gln	Leu	Glu	Ile	Tyr	Lys	Thr	Ile	Gln	Ser	Leu	Pro															
245			250				255																							
aag	gcc	ctg	cag	gaa	cgc	tac	ctg	ctt	gtg	cgc	tat	gag	gac	ctg	gct	932														
Lys	Ala	Leu	Gln	Glu	Arg	Tyr	Leu	Leu	Val	Arg	Tyr	Glu	Asp	Leu	Ala															
260		265				270																								
cga	gcc	cct	gtg	gcc	cag	act	tcc	cga	atg	tat	gaa	ttc	gtg	gga	ttg	980														
Arg	Ala	Pro	Val	Ala	Gln	Thr	Ser	Arg	Met	Tyr	Glu	Phe	Val	Gly	Leu															
275		280				285				290																				
gaa	ttc	ttg	ccc	cat	ctt	cag	acc	tgg	gtg	cat	aac	atc	acc	cga	ggc	1028														

Glu Phe Leu Pro His Leu Gln Thr Trp Val His Asn Ile Thr Arg Gly
 295 300 305

aag ggc atg ggt gac cac gct ttc cac aca aat gcc agg gat gcc ctt 1076
 Lys Gly Met Gly Asp His Ala Phe His Thr Asn Ala Arg Asp Ala Leu
 310 315 320

aat gtc tcc cag gct tgg cgc tgg tct ttg ccc tat gaa aag gtt tct 1124
 Asn Val Ser Gln Ala Trp Arg Trp Ser Leu Pro Tyr Glu Lys Val Ser
 325 330 335

cga ctt cag aaa gcc tgt ggc gat gcc atg aat ttg ctg ggc tac cgc 1172
 Arg Leu Gln Lys Ala Cys Gly Asp Ala Met Asn Leu Leu Gly Tyr Arg
 340 345 350

cac gtc aga tct gaa caa gaa cag aga aac ctg ttg ctg gat ctt ctg 1220
 His Val Arg Ser Glu Gln Glu Gln Arg Asn Leu Leu Leu Asp Leu Leu
 355 360 365 370

tct acc tgg act gtc cct gag caa atc cac taagagggtt gagaaggctt 1270
 Ser Thr Trp Thr Val Pro Glu Gln Ile His
 375 380

tgctgccacc tgggtgcagc ctccagtcact ttctctgaat gcttctgagc cttgcctaca 1330
 tct 1333

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<211> 380

<212> PRT

<213> Homo Sapien

<400> 6

Met Lys Leu Leu Leu Phe Leu Val Ser Gln Met Ala Ile Leu Ala Leu
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Phe Phe His Met Tyr Ser His Asn Ile Ser Ser Leu Ser Met Lys Ala
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Gln Pro Glu Arg Met His Val Leu Val Leu Ser Ser Trp Arg Ser Gly
 35 40 45

Ser Ser Phe Val Gly Gln Leu Phe Gly Gln His Pro Asp Val Phe Tyr
 50 55 60

Leu Met Glu Pro Ala Trp His Val Trp Met Thr Phe Lys Gln Ser Thr
 65 70 75 80

Ala Trp Met Leu His Met Ala Val Arg Asp Leu Ile Arg Ala Val Phe
 85 90 95

Leu Cys Asp Met Ser Val Phe Asp Ala Tyr Met Glu Pro Gly Pro Arg
 100 105 110

Arg Gln Ser Ser Leu Phe Gln Trp Glu Asn Ser Arg Ala Leu Cys Ser
 115 120 125

Ala Pro Ala Cys Asp Ile Ile Pro Gln Asp Glu Ile Ile Pro Arg Ala
 130 135 140

His Cys Arg Leu Leu Cys Ser Gln Gln Pro Phe Glu Val Val Glu Lys
 145 150 155 160

Ala Cys Arg Ser Tyr Ser His Val Val Leu Lys Glu Val Arg Phe Phe
 165 170 175

Asn Leu Gln Ser Leu Tyr Pro Leu Leu Lys Asp Pro Ser Leu Asn Leu


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ccctagccag aggt atg cgg cta ccc cgt ttc tcc agc act gtc atg ctt 110
          Met Arg Leu Pro Arg Phe Ser Ser Thr Val Met Leu
              1              5              10

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Ser Leu Leu Met Val Gln Thr Gly Ile Leu Val Phe Leu Val Ser Arg
          15              20              25

caa gtg cca tcg tcc cca gca ggc ctt ggg gag cgt gtg cac gtg ctg 206
Gln Val Pro Ser Ser Pro Ala Gly Leu Gly Glu Arg Val His Val Leu
          30              35              40

gta ctg tcc tcg tgg cgc tcg ggc tcg tcc ttc gtg ggc cag ctc ttc 254
Val Leu Ser Ser Trp Arg Ser Gly Ser Ser Phe Val Gly Gln Leu Phe
          45              50              55              60

agc caa cac ccc gat gtc ttc tac ctg atg gag ccg gct tgg cac gtc 302

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Ser	Gln	His	Pro	Asp	Val	Phe	Tyr	Leu	Met	Glu	Pro	Ala	Trp	His	Val	
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Trp	Asp	Thr	Leu	Ser	Gln	Gly	Ser	Ala	Pro	Ala	Leu	His	Met	Ala	Val	
			80					85					90			
cgt	gac	ctg	atc	cgc	tca	gtg	ttc	cta	tgc	gac	atg	gac	gta	ttt	gat	398
Arg	Asp	Leu	Ile	Arg	Ser	Val	Phe	Leu	Cys	Asp	Met	Asp	Val	Phe	Asp	
		95					100					105				
gcc	tac	ctg	ccc	tgg	cgc	cgc	aac	atc	tcg	gat	ctc	ttc	cag	tgg	gcg	446
Ala	Tyr	Leu	Pro	Trp	Arg	Arg	Asn	Ile	Ser	Asp	Leu	Phe	Gln	Trp	Ala	
	110						115				120					
gtg	agc	cgc	gca	ttg	tgc	tca	cct	ccg	gtc	tgc	gaa	gcc	ttc	gct	cgt	494
Val	Ser	Arg	Ala	Leu	Cys	Ser	Pro	Pro	Val	Cys	Glu	Ala	Phe	Ala	Arg	
125					130					135					140	
ggc	aac	atc	agc	agc	gag	gag	gtg	tgt	aag	cct	ctg	tgc	gca	acg	cgg	542
Gly	Asn	Ile	Ser	Ser	Glu	Glu	Val	Cys	Lys	Pro	Leu	Cys	Ala	Thr	Arg	
				145					150					155		
ccc	ttc	ggc	ctg	gct	cag	gaa	gcc	tgc	agc	tcc	tat	agt	cac	gtc	gtg	590
Pro	Phe	Gly	Leu	Ala	Gln	Glu	Ala	Cys	Ser	Ser	Tyr	Ser	His	Val	Val	
			160					165					170			
ctc	aag	gag	gtg	cgc	ttc	ttt	aac	cta	cag	gtg	ctc	tac	ccg	ctg	ctc	638
Leu	Lys	Glu	Val	Arg	Phe	Phe	Asn	Leu	Gln	Val	Leu	Tyr	Pro	Leu	Leu	
		175					180					185				
agc	gac	cct	gcg	ctc	aac	ctg	cgc	atc	gtg	cac	cta	gtg	cgc	gac	ccg	686
Ser	Asp	Pro	Ala	Leu	Asn	Leu	Arg	Ile	Val	His	Leu	Val	Arg	Asp	Pro	
	190					195					200					
cgg	gcc	gtg	ctg	cgc	tcc	cga	gag	cag	aca	gcc	aag	gcg	ctg	gca	cgg	734
Arg	Ala	Val	Leu	Arg	Ser	Arg	Glu	Gln	Thr	Ala	Lys	Ala	Leu	Ala	Arg	
205					210					215					220	
gac	aat	ggc	atc	gtc	ctg	ggg	acc	aac	ggc	acg	tgg	gtg	gag	gcg	gac	782
Asp	Asn	Gly	Ile	Val	Leu	Gly	Thr	Asn	Gly	Thr	Trp	Val	Glu	Ala	Asp	
			225						230					235		
ccc	cgg	ctg	cgc	gtg	gtc	aac	gag	gta	tgc	cgc	agc	cat	gtg	cgc	atc	830
Pro	Arg	Leu	Arg	Val	Val	Asn	Glu	Val	Cys	Arg	Ser	His	Val	Arg	Ile	
			240					245					250			
gca	gag	gca	gcc	ttg	cac	aag	ccg	ccg	ccc	ttc	ttg	caa	gat	cgc	tac	878
Ala	Glu	Ala	Ala	Leu	His	Lys	Pro	Pro	Pro	Phe	Leu	Gln	Asp	Arg	Tyr	
	255						260					265				
cgc	ctg	gtg	cgc	tac	gag	gat	ctg	gcc	cgg	gac	cca	ctc	acc	gta	atc	926
Arg	Leu	Val	Arg	Tyr	Glu	Asp	Leu	Ala	Arg	Asp	Pro	Leu	Thr	Val	Ile	
	270					275					280					

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cgt gaa ctc tat gcc ttc acc ggc ctg ggt ctc acg ccg cag ctc cag 974
Arg Glu Leu Tyr Ala Phe Thr Gly Leu Gly Leu Thr Pro Gln Leu Gln
285                290                295                300

act tgg atc cac aat atc acg cat ggt tca ggg cca ggc gcg cgc cgt 1022
Thr Trp Ile His Asn Ile Thr His Gly Ser Gly Pro Gly Ala Arg Arg
305                310                315

gaa gcc ttc aag acc aca tcc agg gat gcg ctc agt gta tcc cag gcc 1070
Glu Ala Phe Lys Thr Thr Ser Arg Asp Ala Leu Ser Val Ser Gln Ala
320                325                330

tgg cgc cac acg ctg ccc ttt gcc aag att cgc cgg gtc cag gaa ctg 1118
Trp Arg His Thr Leu Pro Phe Ala Lys Ile Arg Arg Val Gln Glu Leu
335                340                345

tgc ggg ggt gca ctg cag ctg ctg ggt tac cgg tct gtg cat tcg gag 1166
Cys Gly Gly Ala Leu Gln Leu Leu Gly Tyr Arg Ser Val His Ser Glu
350                355                360

ctt gag caa agg gac ctc tct ctg gac ctc ctg ctg cca aga ggc atg 1214
Leu Glu Gln Arg Asp Leu Ser Leu Asp Leu Leu Leu Pro Arg Gly Met
365                370                375                380

gac agt ttc aag tgg gca tcg tcc acg gag aag caa ccg gaa tct 1259
Asp Ser Phe Lys Trp Ala Ser Ser Thr Glu Lys Gln Pro Glu Ser
385                390                395

tagaatttta gtggagagac ccagctataa cattaggggtc tattggagta tgataaagaa 1319
ggggcttgga gaacccaaaa gcaagtagct gggagtgtga gtgatcttgt cctgtactag 1379
gaaaggatgg agtccaaatc ccacatctct ttctgtccag attgtagttt tcgggttttg 1439
tcttttaggg tttggattcc caccaagtac tatcgaatgg aaagcaaaag ctgtgcccac 1499
ttccttcaga gaggcagcca gcctcctact aaagcaattc ctttctcggt gactctctcc 1559
cctctttgat cataccatgc aatcgacagag aatgggggtcc caggcctgct ctggagtgcg 1619
ggaaaaggcgc ggctgtgggc tggctcctaa aatctgtgca cctgcctctc gttggctcac 1679
ccagacctct gctcactgcc acgccttagt atctcagtc atcatagact tggacagtta 1739
tgggcctggt caaggaggaa aatgagacga tgcttccttc tgtgattctc tgctgacct 1799
tctagaaggg aatccaggca cacacacaac catacctgag gaggatggct ttttaatgaa 1859
tctttgatgt gtcctgagat gaaagatcct aatttatgga aataaacata aatatgctgc 1919
gtgatccaaa aaaaaaaaaa 1937

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<210> 8

<211> 395

<212> PRT

<213> Mus musculus

<400> 8

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Met Arg Leu Pro Arg Phe Ser Ser Thr Val Met Leu Ser Leu Leu Met
1      5      10      15
Val Gln Thr Gly Ile Leu Val Phe Leu Val Ser Arg Gln Val Pro Ser
20      25      30
Ser Pro Ala Gly Leu Gly Glu Arg Val His Val Leu Val Leu Ser Ser
35      40      45
Trp Arg Ser Gly Ser Ser Phe Val Gly Gln Leu Phe Ser Gln His Pro
50      55      60

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Asp Val Phe Tyr Leu Met Glu Pro Ala Trp His Val Trp Asp Thr Leu
 65 70 75 80
 Ser Gln Gly Ser Ala Pro Ala Leu His Met Ala Val Arg Asp Leu Ile
 85 90 95
 Arg Ser Val Phe Leu Cys Asp Met Asp Val Phe Asp Ala Tyr Leu Pro
 100 105 110
 Trp Arg Arg Asn Ile Ser Asp Leu Phe Gln Trp Ala Val Ser Arg Ala
 115 120 125
 Leu Cys Ser Pro Pro Val Cys Glu Ala Phe Ala Arg Gly Asn Ile Ser
 130 135 140
 Ser Glu Glu Val Cys Lys Pro Leu Cys Ala Thr Arg Pro Phe Gly Leu
 145 150 155 160
 Ala Gln Glu Ala Cys Ser Ser Tyr Ser His Val Val Leu Lys Glu Val
 165 170 175
 Arg Phe Phe Asn Leu Gln Val Leu Tyr Pro Leu Leu Ser Asp Pro Ala
 180 185 190
 Leu Asn Leu Arg Ile Val His Leu Val Arg Asp Pro Arg Ala Val Leu
 195 200 205
 Arg Ser Arg Glu Gln Thr Ala Lys Ala Leu Ala Arg Asp Asn Gly Ile
 210 215 220
 Val Leu Gly Thr Asn Gly Thr Trp Val Glu Ala Asp Pro Arg Leu Arg
 225 230 235 240
 Val Val Asn Glu Val Cys Arg Ser His Val Arg Ile Ala Glu Ala Ala
 245 250 255
 Leu His Lys Pro Pro Pro Phe Leu Gln Asp Arg Tyr Arg Leu Val Arg
 260 265 270
 Tyr Glu Asp Leu Ala Arg Asp Pro Leu Thr Val Ile Arg Glu Leu Tyr
 275 280 285
 Ala Phe Thr Gly Leu Gly Leu Thr Pro Gln Leu Gln Thr Trp Ile His
 290 295 300
 Asn Ile Thr His Gly Ser Gly Pro Gly Ala Arg Arg Glu Ala Phe Lys
 305 310 315 320
 Thr Thr Ser Arg Asp Ala Leu Ser Val Ser Gln Ala Trp Arg His Thr
 325 330 335
 Leu Pro Phe Ala Lys Ile Arg Arg Val Gln Glu Leu Cys Gly Gly Ala
 340 345 350
 Leu Gln Leu Leu Gly Tyr Arg Ser Val His Ser Glu Leu Glu Gln Arg
 355 360 365
 Asp Leu Ser Leu Asp Leu Leu Leu Pro Arg Gly Met Asp Ser Phe Lys
 370 375 380
 Trp Ala Ser Ser Thr Glu Lys Gln Pro Glu Ser
 385 390 395

<210> 9

<211> 26

<212> DNA

<213> Homo Sapien

<400> 9

ctggctggcc aggatgaagt atctcc

26

<210> 10

<211> 28

<212> DNA

<213> Homo Sapien

<400> 10

cctgatgctg actcagtaga tctgtgtc

28

<210> 11

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 11

cgggatcccg aggcctggc ctggcccact cc

32

<210> 12

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 12

gctctagact cagtagatct gtgtctgatt gc

32

<210> 13

<211> 24

<212> DNA

<213> Homo sapien

<400> 13

ttcctgctgc tggatgacaa gtcc

24

<210> 14

<211> 25

<212> DNA

<213> Homo sapien

<400> 14

caggacctgc ttgagcgtga gggtg

25

<210> 15

<211> 29

<212> DNA

<213> Mus musculus

<400> 15

atggaaatcc cactggagaa tgcgccgt

29

<210> 16

<211> 27

<212> DNA

<213> Mus musculus

<400> 16 gcctgcaaac tatgggcgcc gccagat	27
<210> 17 <211> 26 <212> DNA <213> Homo sapien	
<400> 17 ccgaattctc ccgagaacgc acaaag	26
<210> 18 <211> 27 <212> DNA <213> Homo sapien	
<400> 18 cccaagcttc tcatagcgca caagcag	27
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<210> 22 <211> 22 <212> DNA <213> Homo sapien	
<400> 22 tttggcagat gacctgcatc ac	22
<210> 23 <211> 22 <212> DNA	

<213> Homo sapien

<400> 23

agaacgcaca aaggagatct ca

22

<210> 24

<211> 22

<212> DNA

<213> Homo sapien

<400> 24

agatgtaggc aaggctcaga ag

22

<210> 25

<211> 326

<212> PRT

<213> Mus musculus

<400> 25

Met Ala Ser Lys Val Ser Cys Leu Tyr Val Leu Ser Val Val Cys Trp
 1 5 10 15
 Ala Ser Ala Leu Trp Tyr Leu Ser Ile Thr Arg Pro Thr Ser Ser Tyr
 20 25 30
 Thr Gly Ser Lys Pro Phe Ser His Leu Thr Val Ala Arg Lys Asn Phe
 35 40 45
 Thr Phe Gly Asn Ile Arg Thr Arg Pro Ile Asn Pro His Ser Phe Glu
 50 55 60
 Phe Leu Ile Asn Glu Pro Asn Lys Cys Glu Lys Asn Ile Pro Phe Leu
 65 70 75 80
 Val Ile Leu Ile Ser Thr Thr His Lys Glu Phe Asp Ala Arg Gln Ala
 85 90 95
 Ile Arg Glu Thr Trp Gly Asp Glu Asn Asn Phe Lys Gly Ile Lys Ile
 100 105 110
 Ala Thr Leu Phe Leu Leu Gly Lys Asn Ala Asp Pro Val Leu Asn Gln
 115 120 125
 Met Val Glu Gln Glu Ser Gln Ile Phe His Asp Ile Ile Val Glu Asp
 130 135 140
 Phe Ile Asp Ser Tyr His Asn Leu Thr Leu Lys Thr Leu Met Gly Met
 145 150 155 160
 Arg Trp Val Ala Thr Phe Cys Ser Lys Ala Lys Tyr Val Met Lys Thr
 165 170 175
 Asp Ser Asp Ile Phe Val Asn Met Asp Asn Leu Ile Tyr Lys Leu Leu
 180 185 190
 Lys Pro Ser Thr Lys Pro Arg Arg Tyr Phe Thr Gly Tyr Val Ile
 195 200 205
 Asn Gly Gly Pro Ile Arg Asp Val Arg Ser Lys Trp Tyr Met Pro Arg
 210 215 220
 Asp Leu Tyr Pro Asp Ser Asn Tyr Pro Pro Phe Cys Ser Gly Thr Gly
 225 230 235 240
 Tyr Ile Phe Ser Ala Asp Val Ala Glu Leu Ile Tyr Lys Thr Ser Leu
 245 250 255
 His Thr Arg Leu Leu His Leu Glu Asp Val Tyr Val Gly Leu Cys Leu
 260 265 270
 Arg Lys Leu Gly Ile His Pro Phe Gln Asn Ser Gly Phe Asn His Trp
 275 280 285

Lys Met Ala Tyr Ser Leu Cys Arg Tyr Arg Arg Val Ile Thr Val His
 290 295 300
 Gln Ile Ser Pro Glu Glu Met His Arg Ile Trp Asn Asp Met Ser Ser
 305 310 315 320
 Lys Lys His Leu Arg Cys
 325

<210> 26

<211> 422

<212> PRT

<213> Mus musculus

<400> 26

Met Leu Gln Trp Arg Arg Arg His Cys Cys Phe Ala Lys Met Thr Trp
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 Ser Pro Lys Arg Ser Leu Leu Arg Thr Pro Leu Thr Gly Val Leu Ser
 20 25 30
 Leu Val Phe Leu Phe Ala Met Phe Leu Phe Phe Asn His His Asp Trp
 35 40 45
 Leu Pro Gly Arg Pro Gly Phe Lys Glu Asn Pro Val Thr Tyr Thr Phe
 50 55 60
 Arg Gly Phe Arg Ser Thr Lys Ser Glu Thr Asn His Ser Ser Leu Arg
 65 70 75 80
 Thr Ile Trp Lys Glu Val Ala Pro Gln Thr Leu Arg Pro His Ile Ala
 85 90 95
 Ser Asn Ser Ser Asn Thr Glu Leu Ser Pro Gln Gly Val Thr Gly Leu
 100 105 110
 Gln Asn Thr Leu Ser Ala Asn Gly Ser Ile Tyr Asn Glu Lys Gly Thr
 115 120 125
 Gly His Pro Asn Ser Tyr His Phe Lys Tyr Ile Ile Asn Glu Pro Glu
 130 135 140
 Lys Cys Gln Glu Lys Ser Pro Phe Leu Ile Leu Leu Ile Ala Ala Glu
 145 150 155 160
 Pro Gly Gln Ile Glu Ala Arg Arg Ala Ile Arg Gln Thr Trp Gly Asn
 165 170 175
 Glu Thr Leu Ala Pro Gly Ile Gln Ile Ile Arg Val Phe Leu Leu Gly
 180 185 190
 Ile Ser Ile Lys Leu Asn Gly Tyr Leu Gln His Ala Ile Gln Glu Glu
 195 200 205
 Ser Arg Gln Tyr His Asp Ile Ile Gln Gln Glu Tyr Leu Asp Thr Tyr
 210 215 220
 Tyr Asn Leu Thr Ile Lys Thr Leu Met Gly Met Asn Trp Val Ala Thr
 225 230 235 240
 Tyr Cys Pro His Thr Pro Tyr Val Met Lys Thr Asp Ser Asp Met Phe
 245 250 255
 Val Asn Thr Glu Tyr Leu Ile His Lys Leu Leu Lys Pro Asp Leu Pro
 260 265 270
 Pro Arg His Asn Tyr Phe Thr Gly Tyr Leu Met Arg Gly Tyr Ala Pro
 275 280 285
 Asn Arg Asn Lys Asp Ser Lys Trp Tyr Met Pro Pro Asp Leu Tyr Pro
 290 295 300
 Ser Glu Arg Tyr Pro Val Phe Cys Ser Gly Thr Gly Tyr Val Phe Ser
 305 310 315 320
 Gly Asp Leu Ala Glu Lys Ile Phe Lys Val Ser Leu Gly Ile Arg Arg


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          325          330          335
Leu His Leu Glu Asp Val Tyr Val Gly Ile Cys Leu Ala Lys Leu Arg
          340          345          350
Val Asp Pro Val Pro Pro Asn Glu Phe Val Phe Asn His Trp Arg
          355          360          365
Val Ser Tyr Ser Ser Cys Lys Tyr Ser His Leu Ile Thr Ser His Gln
          370          375          380
Phe Gln Pro Ser Glu Leu Ile Lys Tyr Trp Asn His Leu Gln Gln Asn
          385          390          395          400
Lys His Asn Ala Cys Ala Asn Ala Ala Lys Glu Lys Ala Gly Arg Tyr
          405          410          415
Arg His Arg Lys Leu His
          420

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<210> 27

<211> 331

<212> PRT

<213> Mus musculus

<400> 27

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Met Ala Pro Ala Val Leu Thr Ala Leu Pro Asn Arg Met Ser Leu Arg
  1          5          10          15
Ser Leu Lys Trp Ser Leu Leu Leu Leu Ser Leu Leu Ser Phe Leu Val
          20          25          30
Ile Trp Tyr Leu Ser Leu Pro His Tyr Asn Val Ile Glu Arg Val Asn
          35          40          45
Trp Met Tyr Phe Tyr Glu Tyr Glu Pro Ile Tyr Arg Gln Asp Phe Arg
          50          55          60
Phe Thr Leu Arg Glu His Ser Asn Cys Ser His Gln Asn Pro Phe Leu
          65          70          75          80
Val Ile Leu Val Thr Ser Arg Pro Ser Asp Val Lys Ala Arg Gln Ala
          85          90          95
Ile Arg Val Thr Trp Gly Glu Lys Lys Ser Trp Trp Gly Tyr Glu Val
          100          105          110
Leu Thr Phe Leu Leu Gly Gln Ala Glu Arg Glu Asp Lys Thr
          115          120          125
Leu Ala Leu Ser Leu Glu Asp Glu His Val Leu Tyr Gly Asp Ile Ile
          130          135          140
Arg Gln Asp Phe Leu Asp Thr Tyr Asn Asn Leu Thr Leu Lys Thr Ile
          145          150          155          160
Met Ala Phe Arg Trp Val Met Glu Phe Cys Pro Asn Ala Lys Tyr Ile
          165          170          175
Met Lys Thr Asp Thr Asp Val Phe Ile Asn Thr Gly Asn Leu Val Lys
          180          185          190
Tyr Leu Leu Asn Leu Asn His Ser Glu Lys Phe Phe Thr Gly Tyr Pro
          195          200          205
Leu Ile Asp Asn Tyr Ser Tyr Arg Gly Phe Phe His Lys Asn His Ile
          210          215          220
Ser Tyr Gln Glu Tyr Pro Phe Lys Val Phe Pro Pro Tyr Cys Ser Gly
          225          230          235          240
Leu Gly Tyr Ile Met Ser Gly Asp Leu Val Pro Arg Val Tyr Glu Met
          245          250          255
Met Ser His Val Lys Pro Ile Lys Phe Glu Asp Val Tyr Val Gly Ile
          260          265          270

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Cys Leu Asn Leu Leu Lys Val Asp Ile His Ile Pro Glu Asp Thr Asn
 275 280 285
 Leu Phe Phe Leu Tyr Arg Ile His Leu Asp Val Cys Gln Leu Arg Arg
 290 295 300
 Val Ile Ala Ala His Gly Phe Ser Ser Lys Glu Ile Ile Thr Phe Trp
 305 310 315 320
 Gln Val Met Leu Arg Asn Thr Thr Cys His Tyr
 325 330

<210> 28

<211> 371

<212> PRT

<213> Mus musculus

<400> 28

Met Pro Leu Ser Leu Phe Arg Arg Val Leu Leu Ala Val Leu Leu Leu
 1 5 10 15
 Val Ile Ile Trp Thr Leu Phe Gly Pro Ser Gly Leu Gly Glu Glu Leu
 20 25 30
 Leu Ser Leu Ser Leu Ala Ser Leu Leu Pro Ala Pro Ala Ser Pro Gly
 35 40 45
 Pro Pro Leu Ala Leu Pro Arg Leu Leu Ile Ser Asn Ser His Ala Cys
 50 55 60
 Gly Gly Ser Gly Pro Pro Pro Phe Leu Leu Ile Leu Val Cys Thr Ala
 65 70 75 80
 Pro Glu His Leu Asn Gln Arg Asn Ala Ile Arg Ala Ser Trp Gly Ala
 85 90 95
 Ile Arg Glu Ala Arg Gly Phe Arg Val Gln Thr Leu Phe Leu Leu Gly
 100 105 110
 Lys Pro Arg Arg Gln Gln Leu Ala Asp Leu Ser Ser Glu Ser Ala Ala
 115 120 125
 His Arg Asp Ile Leu Gln Ala Ser Phe Gln Asp Ser Tyr Arg Asn Leu
 130 135 140
 Thr Leu Lys Thr Leu Ser Gly Leu Asn Trp Val Asn Lys Tyr Cys Pro
 145 150 155 160
 Met Ala Arg Tyr Ile Leu Lys Thr Asp Asp Asp Val Tyr Val Asn Val
 165 170 175
 Pro Glu Leu Val Ser Glu Leu Ile Gln Arg Gly Gly Pro Ser Glu Gln
 180 185 190
 Trp Gln Lys Gly Lys Glu Ala Gln Glu Glu Thr Thr Ala Ile His Glu
 195 200 205
 Glu His Arg Gly Gln Ala Val Pro Leu Leu Tyr Leu Gly Arg Val His
 210 215 220
 Trp Arg Val Arg Pro Thr Arg Thr Pro Glu Ser Arg His His Val Ser
 225 230 235 240
 Glu Glu Leu Trp Pro Glu Asn Trp Gly Pro Phe Pro Pro Tyr Ala Ser
 245 250 255
 Gly Thr Gly Tyr Val Leu Ser Ile Ser Ala Val Gln Leu Ile Leu Lys
 260 265 270
 Val Ala Ser Arg Ala Pro Pro Leu Pro Leu Glu Asp Val Phe Val Gly
 275 280 285
 Val Ser Ala Arg Arg Gly Gly Leu Ala Pro Thr His Cys Val Lys Leu
 290 295 300
 Ala Gly Ala Thr His Tyr Pro Leu Asp Arg Cys Cys Tyr Gly Lys Phe

305					310					315				320	
Leu	Leu	Thr	Ser	His	Lys	Val	Asp	Pro	Trp	Gln	Met	Gln	Glu	Ala	Trp
				325					330					335	
Lys	Leu	Val	Ser	Gly	Met	Asn	Gly	Glu	Arg	Thr	Ala	Pro	Phe	Cys	Ser
			340					345					350		
Trp	Leu	Gln	Gly	Phe	Leu	Gly	Thr	Leu	Arg	Cys	Arg	Phe	Ile	Ala	Trp
		355					360					365			
Phe	Ser	Ser													
		370													

<210> 29

<211> 325

<212> PRT

<213> Mus musculus

<400> 29

Met	Lys	Val	Phe	Arg	Arg	Ala	Trp	Arg	His	Arg	Val	Ala	Leu	Gly	Leu
1				5					10					15	
Gly	Gly	Leu	Ala	Phe	Cys	Gly	Thr	Thr	Leu	Leu	Tyr	Leu	Ala	Arg	Cys
		20					25					30			
Ala	Ser	Glu	Gly	Glu	Thr	Pro	Ser	Ala	Ser	Gly	Ala	Ala	Arg	Pro	Arg
	35					40					45				
Ala	Lys	Ala	Phe	Leu	Ala	Val	Leu	Val	Ala	Ser	Ala	Pro	Arg	Ala	Val
	50				55						60				
Glu	Arg	Arg	Thr	Ala	Val	Arg	Ser	Thr	Trp	Leu	Ala	Pro	Glu	Arg	Arg
65				70					75					80	
Gly	Gly	Pro	Glu	Asp	Val	Trp	Ala	Arg	Phe	Ala	Val	Gly	Thr	Gly	Gly
			85					90					95		
Leu	Gly	Ser	Glu	Glu	Arg	Arg	Ala	Leu	Glu	Leu	Glu	Gln	Ala	Gln	His
		100					105					110			
Gly	Asp	Leu	Leu	Leu	Leu	Pro	Ala	Leu	Arg	Asp	Ala	Tyr	Glu	Asn	Leu
	115					120					125				
Thr	Ala	Lys	Val	Leu	Ala	Met	Leu	Thr	Trp	Val	Asp	Glu	Arg	Val	Asp
	130					135					140				
Phe	Glu	Phe	Val	Ile	Lys	Ala	Asp	Asp	Asp	Ser	Phe	Ala	Arg	Leu	Asp
145				150					155					160	
Ala	Ile	Leu	Val	Asp	Leu	Arg	Ala	Arg	Glu	Pro	Ala	Arg	Arg	Arg	Arg
			165					170					175		
Leu	Tyr	Trp	Gly	Phe	Phe	Ser	Gly	Arg	Gly	Arg	Val	Lys	Pro	Gly	Gly
		180					185					190			
Arg	Trp	Arg	Glu	Ala	Ala	Trp	Gln	Leu	Cys	Asp	Tyr	Tyr	Leu	Pro	Tyr
	195					200					205				
Ala	Leu	Gly	Gly	Gly	Tyr	Val	Leu	Ser	Ala	Asp	Leu	Val	His	Tyr	Ile
	210				215						220				
Arg	Leu	Ser	Arg	Glu	Tyr	Leu	Arg	Ala	Trp	His	Ser	Glu	Asp	Val	Ser
225				230					235					240	
Leu	Gly	Thr	Trp	Leu	Ala	Pro	Val	Asp	Val	Gln	Arg	Glu	His	Asp	Pro
			245					250					255		
Arg	Phe	Asp	Thr	Glu	Tyr	Lys	Ser	Arg	Gly	Cys	Asn	Asn	Gln	Tyr	Leu
		260					265					270			
Val	Thr	His	Lys	Gln	Ser	Pro	Glu	Asp	Met	Leu	Glu	Lys	Gln	Gln	Met
	275					280					285				
Leu	Leu	His	Glu	Gly	Arg	Leu	Cys	Lys	His	Glu	Val	Gln	Leu	Arg	Leu
	290					295					300				

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Ser	Tyr	Val	Tyr	Asp	Trp	Ser	Ala	Pro	Pro	Ser	Gln	Cys	Cys	Gln	Arg
305					310					315					320
Lys	Glu	Gly	Val	Pro											
				325											

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